Good practice report

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Measuring the mass of an electron: an undergraduate laboratory experiment with high resolution mass spectrometry

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Abstract: High-resolution mass spectrometry (HRMS) has become increasingly affordable and user-friendly. Its potential spans a wide range of applications and experiments including the measurement of accurate masses, supporting the elucidation of elemental compositions and the identification of unknown compounds. To illustrate the main features of mass spectrometry, and particularly, of HRMS, we have designed and implemented a 3-h laboratory experiment using direct infusion electrospray HRMS analysis of non-steroidal anti-inflammatory drugs (e.g., ibuprofen or naproxen) solutions, acquiring full-scan spectra in both positive and negative ionization modes. The experimental accurate mass measurements (m/z values) of selected characteristic fragment ions -so called twin ions, with common elemental composition in both ionization modes but with different charge, allow the indirect measurement of the mass of an electron with relative errors below 5% with respect to the accepted IUPAC value (0.00055 Da). The experiment demonstrates how powerful and useful HRMS can be for research challenges often encountered during undergraduate or graduate research projects as well as for addressing undergraduate level general chemistry problems that provide the opportunity to discuss aspects related to the Nature of Science in an analytical chemistry context (such as measurement precision and accuracy).

Keywords: analytical chemistry; instrumental application; laboratory activity; mass spectrometry.

Introduction

The empirical determination of a molecular formula is pivotal in structure elucidation in organic chemistry laboratories. It can be accomplished with High Resolution Mass Spectrometry (HRMS) (Marshall & Hendrickson, 2008; Xian, Hendrickson, & Marshall, 2012) which refers to the ability of a mass spectrometer to provide a specified value of m/z beyond integral numbers up to 4-5 decimal places. This, together with the mass defect of stable isotopes, makes possible the elucidation of the elemental compositions of species of interest for relatively low molecular weight values.

In the 70–90s, HRMS was limited to magnetic sector or Fourier Transform Ion Cyclotron Resonance (FTICR) instruments. Both types have the liability of having a reputation of being instruments which can be difficult to run and maintain. Yet, FTICR still remains as the technology offering the best performance in terms of mass accuracy and resolution, featuring values well above 3,000,000, enabling the measurement

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of peptides with theoretical mass differences lower than the mass of an electron (ca. 0.0005 Da) (He, Hendrickson, & Marshall, 2001). Whereas magnetic sectors are no longer in the landscape for the analysis of organic compounds-except for selected applications such as dioxin analysis-, FT-ICR instruments are still being operated, although only for highly specialized research applications where such high resolving power are demanded (Marshall & Hendrickson, 2008; Xian et al., 2012). The last two decades have witnessed an unprecedented growth of HRMS (Krajewski, Rodgers, & Marshall, 2017). Several vendors have been commercializing upgraded benchtop versions of orbitrap and time-of-flight (TOF) instruments, with improvements that broadened the range of applications and enabled more versatile and user-friendly operation.

As a stand-alone technique or in combination with chromatography, HRMS is becoming a central technique in most laboratories. For instance, HRMS or accurate-mass MS is of the utmost importance for both synthesis and analysis steps in chemical laboratories: from the elucidation of the elemental composition of an unknown, to the confirmation of a finding in a sample or the identification of reaction impurities or intermediates, or for reporting structural data of a newly synthesized species. Recent articles have reported the successful incorporation of mass spectrometry (Bain, Pulliam, Raab, & Cooks, 2015, 2016; Beck & Erdmann, 2018; Mendes, Ramamurthy, & Da Silva, 2015; Sneha, Dulay, & Zare, 2017; Stock & March, 2014), gas chromatography/mass spectrometry (GC-MS) (Giarikos, Patel, Lister & Razeghifad, 2013; Pact, Lee, Chin, & Marriot, 2016) and liquid chromatography/mass spectrometry (LC-MS) (Betts & Palkendo, 2018; Pacot, Lee, Chin, & Marriott, 2016; Parker, Beers, & Vergne, 2017;) in both undergraduate and graduate courses. These reports uncover MS to students by proposing laboratory practices ranging from fundamental courses for electrospray and MS principles understanding to something as intricate as monitoring on-line reactions *in situ* by using mass spectrometry instrumentation. Although recommended by multiple educational publications (Alty & LaRiviere, 2016; Stock, 2017; Walsh, Ashe, & Walsh, 2012), the implementation of undergraduate and graduate laboratory courses using high-end mass spectrometers need further development. These courses are scarcely included in college laboratory curricula (Boyce, Lawler, Tu, & Reinke, 2019; Doucette & Chisholm, 2019).

With this in mind, and to reinforce the theoretical lectures of mass spectrometry, we have designed and implemented a streamlined mass spectrometry laboratory experiment (<10 min of data acquisition) that ends up allowing the measurement of the mass of the electron, and also addresses several pillars of mass spectral interpretation. The experiment constitutes a simple demonstration of the utility of HRMS to cope with topics beyond its classical applications at the undergraduate level (i.e., structure elucidation, sample identification/quantification, reaction monitoring, etc.) and aims at promoting its incorporation in upper-division undergraduate chemistry courses. The goal is to measure the mass of the electron while getting familiar with the operation of a high-resolution mass spectrometer and to the output (spectra) typically obtained. The mass of the electron has been subject of study through various experiments throughout history, among others, is the charge-to-mass relationship of the electron discovered by Thomson (1897). The first determination of its mass was undertaken by Millikan with the camera fog and subsequently all those experiments were carried out to improve the accuracy of said value. In particular, the proposed approach is based on the formation of the so-called twin ions (Bowie, 1984; Ferrer & Thurman, 2005; Scheweikhard, Drader, Shi, Hendrickson, & Marshall, 2002). These species, which are formed in drugs such as ibuprofen, diclofenac, naproxen, or ketoprofen, yield characteristic ions with the opposite charge but with the same elemental composition. The ions have a difference of two electrons, which can be experimentally measured by HRMS using time-of-flight, FT-ICR, or orbitrap instruments (Bowie, 1984; Ferrer & Thurman, 2005; Scheweikhard, Drader, Shi, Hendrickson, & Marshall, 2002).

The described experiments encompass many relevant concepts related to mass spectrometry in particular and chemistry in general. Aspects such as stable isotopes and isotope abundances, isotopic fine structure, monoisotopic mass, average mass and nominal mass, the concept of accurate mass measurements, resolving power in mass spectrometry, the effect of the actual m/z value on resolving power

measurements, relative mass errors and its use in confirming elemental composition are addressed. In addition, it also offers the opportunity to discuss aspects directly related to the History and Nature of Science concerning: the characteristics of the different experiments to measure the mass of the electron, the nature of the involved scientific processes, and the accuracy and precision of the different measurements accomplished over history. After the session, the student calculates the exact mass associated to elemental compositions using freely available elemental composition calculator tools, identify mass defects, and understand the nitrogen rule and the double bond and ring equivalent number. Besides MS theory, they get familiar with the main features of electrospray ionization (ESI) spectra, the interpretation of typical fragmentation in even electron mass spectra (ESI), the use of specialized software for acquisition and data processing in mass spectrometry. During the course, the students gained hands-on-experience on freely available software (molecular weight calculator) or web-based molecular weight or isotopic distribution calculators (see Supplementary Material). Therefore, the laboratory class described illustrates the potential of mass spectrometry in general, and of HRMS to address many challenges that students may tackle with on their research projects at either undergraduate or graduate level.

Rationale of the experiment

The capability of non-steroidal anti-inflammatory drugs (NSAIDs) to ionize in both positive and negative ion mode, plus the unique feature of forming the so-called twin ions (Ferrer & Thurman, 2005), makes them a perfect target analyte for the proposed studies. Twin ions have the same elemental composition, but different charge (positive and negative), and consequently their masses differ in two electrons. The twin ions are fragment ions obtained from the neutral loss of CO_2 (44 Da) in the negative ion mode or HCOOH (46 Da) in the positive ion mode after collision induced dissociation (CID) experiments. Table 1 summarizes the list of species formed and elemental compositions for four different NSAID species. The CID can be accomplished either with or without precursor ion isolation or even with in-source CID experiments. For the sake of simplicity and due to the lability of the NSAID and easiness to form the twin ions, we used in-source CID harnessing the acceleration voltages applied to the first vacuum stages ion lenses and ion transmission regions in the MS instrument. The use of in-source CID allows the implementation of this experiment to any high-resolution mass spectrometer (even single stage HRMS) with atmospheric pressure sampling (e.g., operated with electrospray ionization). If a tandem MS instrument is available, like in the case of Q-Exactive orbitrap, CID MS/MS experiments may also be conducted for targeted species fragmentation involving precursor ion isolation.

Experiments planned

The experiment consists of the following steps: (i) preparation of NSAIDs standard solutions from a stock solution of e.g. 500 g mL^{-1} already prepared by the instructor; (ii) calibration, in both polarity modes, of the m/z axis using manufacturer specifications and tune/calibration solutions, supervised by the instructor; (iii) direct infusion ESI-HRMS using in-source CID fragmentation or related CID approaches and acquisition with polarity switching every 30 s; and, (iv) interpretation of results. The experiment is designed for a 3-h laboratory period, for a group of up to four students. Students typically work in groups of two. Approximately 30 min are assigned to prepare sample solutions for HRMS analysis. The experiment acquisition time is around 10 min, including the preparation of the infusion and six replicates of acquisition of mass spectra for 30 s in each ionization mode (ca. 6 min). The rest of the time planned is to process and interpret the data, calculate the average mass of the electron from the different experiments and, read carefully and respond to the questions proposed.

The actual data shown were obtained using a Thermo Q-Exactive Orbitrap mass spectrometer (Thermo Fischer Scientific, San José, CA, USA) equipped with a quadrupole and a higher-energy collisional dissociation (HCD) cell, and an electrospray standard electrospray ionization source (IonMax™, Thermo Scientific) with a syringe infusion pump available for standard tuning and mass calibration procedures (Harvard Apparatus, Holliston, MA, USA). The details of the mass spectrometer acquisition mode and parameters selected are shown in Supplementary Material. Individual standards solutions of the different pharmaceuticals (Sigma-Aldrich) were prepared in methanol at 500 g mL⁻¹ and were further diluted with 50:50 (v/v) MeOH:H₂O, with 0.1% formic acid (Sigma-Aldrich).

Hazards

The experiment should be performed under the supervision of a laboratory instructor. The student should follow instructions when handling and operating the high-voltage ionization source of the mass spectrometer and wear protective clothing and eyewear in the lab.

Table 1: Theoretical masses of the selected spectral features of potentially selected NSAIDs that can be used in the experiment.

Compound	Structure	Elemental Formula	Exact mass	Twin-ion in Positive ion mode	Twin-ion in Negative ion mode
Ibuprofen		C ₁₃ H ₁₈ O ₂	206.1307
				[C ₁₂ H ₁₇ O] ⁺ Exact Mass:161.1325	[C ₁₂ H ₁₇ O] ⁻ Exact Mass:161.1336
Naproxen		C ₁₄ H ₁₄ O ₃	230.0943
				[C ₁₃ H ₁₃ O] ⁺ Exact Mass:185.0961	[C ₁₃ H ₁₃ O] ⁻ Exact Mass:185.0972
Ketoprofen		C ₁₆ H ₁₄ O ₃	254.0943
				[C ₁₅ H ₁₃ O] ⁺ Exact Mass:209.0961	[C ₁₅ H ₁₃ O] ⁻ Exact Mass:209.0972
Diclofenac		C ₁₄ H ₁₁ NO ₂ Cl ₂	295.0167
				[C ₁₃ H ₁₀ Cl ₂ N] ⁺ Exact Mass:250.0185	[C ₁₃ H ₁₀ Cl ₂ N] ⁻ Exact Mass:250.0196

Exact mass refers to the theoretical calculated value.

Results and discussion

The first objective is to detect and identify both twin ions for the selected NSAID species as shown in Table 1.

Regardless of the compound and its initial mass, all NSAIDs yielded fragment ions from the loss of formic acid (HCOOH) in the positive ion mode, with the subsequent transfer of charge from the protonated carboxylic group to the benzylic carbon atom remaining. An analogous process takes place in the negative ion mode, this time involving the loss of CO₂, with the remaining negative charge stabilized by the benzylic moiety. The fragment ion formation involves the cleavage of a bond and the charge migration. As usual in ESI, both precursor and product ions are even electron ions, displaying neutral losses of 46 and 44 Da in positive and negative ion mode, respectively. Both fragment ions have the same elemental composition with the difference of two electrons that the negative ion additionally contains. The theoretical mass of an electron is 0.00055 Da, while the theoretical exact mass difference between each two twin ions of 0.0011 Da.

Firstly, after preliminary rinsing of the tubing, the instrument is subjected to standard mass-to-charge axis calibration giving to the student the sense of direct infusion HRMS instrument preparation. Data acquisition using the polarity switching mode took ca. 8 min for diclofenac (Figure 1). The total ion current (TIC) plots to assemble a square waveform, with different plateaus and abundance changes that correspond to the changes in the polarity acquisition mode of electrospray, with the higher abundance of ions, corresponding to positive ionization mode, which typically generates one order of magnitude higher abundances than negative ionization mode (Figure 1). Each polarity step includes at least 30 s of acquisition time corresponding to at least 50 acquisition points. A total of 900 spectra were collected in this example, accounting for ca. 2 Hz frequency when acquiring at an instrument resolving power of 140,000. The targeted m/z , either in positive and negative ion mode, should correspond with the average of at least 10 s of the measurement. At least five replicates should be performed in both polarities; the experimental m/z values should be recorded by the student annotating and calculating the figures showed in Table 2.

Table 2: Data from the experimental measurements of the mass of an electron using HRMS using diclofenac as example.

Analysis #	Theoretical m/z (ESI ⁺)	Theoretical m/z (ESI ⁻)	Experimental m/z (ESI ⁺)	Experimental m/z (ESI ⁻)	($\Delta m/z$)	m/z electron ^a ($\Delta m/z$)/2	Error (%)
1	250.0185	250.0196	250.01850	250.01952	0.00102	0.00051	6.93%
2	250.0185	250.0196	250.01849	250.01954	0.00105	0.00052	4.20%
3	250.0185	250.0196	250.01848	250.01952	0.00104	0.00052	5.11%
4	250.0185	250.0196	250.01850	250.01953	0.00103	0.00052	6.02%
5	250.0185	250.0196	250.01847	250.01951	0.00104	0.00052	5.11%
Mean value	–	–	250.01849	250.01952	0.00104	0.00052	5.47%
Standard deviation	–	–	1.30E-05	1.14E-05	1.14E-05	5.70E-06	1.04E-02
RSD (%)	–	–	–	–	–	1.10%	19.00%

^aReference value of the mass of an electron: 0.000548 Da.

Two adjacent measurements in positive and negative ion mode are used to calculate the experimental value accounting for the mass of two electrons, the actual difference between these twin ions as shown in Figure 1a. The instrumental conditions collected in the Supplementary Material were selected in such a way the (de)protonated molecule was fragmented to yield the fragment ions. Diclofenac yielded unique fragment ions in positive ion mode at m/z 250.0183 and in negative ion mode at m/z 250.0193, whose mass difference enables the calculation of the electron mass (Figure 1b). Among the different features observed in the mass spectra, when working with diclofenac, the student should observe the presence of “A + 2” isotopic signals from the two chlorine atoms in the composition, kept after the neutral loss of CO₂ or HCOOH yielding the

Figure 1: Example of ESI-HRMS measurement of the mass of the electron using diclofenac.

(a) Total ion current (TIC) of the experiment, with different plateaus, positive ion mode the plateau with higher abundances highlighted with a blue dot and negative ion mode with a red circle. (b) Overlapped diclofenac twin ions via ESI-HRMS tandem MS spectra in positive (blue trace) and negative (red trace) ion modes (the same x-axis scale, and the y-axis abundance (x4) in negative ion mode). (c) ESI-HRMS tandem MS spectra in positive ion mode (m/z 250.0183), and (d) ESI-HRMS tandem MS spectra in negative ion mode (m/z 250.01931). The m/z difference is 0.0010 Da, in both positive and negative ion modes, gives an electron mass of 0.00050 Da with a relative error of 8.76%, compared to the accepted value of the mass of the electron by the IUPAC (0.000548 Da). For details, see text.

studied fragment ion. Another feature that the students need to notice is the presence of multiple adducts ions. Even when not added as an additive, sodium tends to form adducts during electrospray ionization in positive ion mode due to its presumable ubiquity (i.e. sodium adduct ions, $[M+Na]^+$, at m/z 318.0057 for diclofenac (Figure 1c).

Conclusions

The proposed laboratory experiment, which can be conducted in a 3-h laboratory session, helps undergraduate and graduate students to gain experience in a field that is not always included during lectures. The

experiment's success relies on the careful mass calibration of the HRMS instrument following manufacturer accurate-mass calibration protocols. Students will have the opportunity to participate and understand the operation of high-end instruments, which later could be the methodology selected for their career path. The present work and protocol were developed to be done in a Q-Exactive Orbitrap, still, the instructions could be easily adjusted for other MS platforms, such as FTICR or Time-of-Flights mass spectrometers, the latter readily available in many university laboratories worldwide. The question and material exposed in the Supplementary Material are more focused on undergraduate student learning levels. Different complementary activities have been designed using open software tools. The activities may need adjustments in terms of content when used with graduate students.

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Supplementary Material: The online version of this article offers supplementary material (<https://doi.org/10.1515/cti-2021-0016>). Laboratory handout, learning objectives, potential exercises/questions, and instrument and software use instructions.