Research Article

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A predictive science approach to aid understanding of electrospray ionisation tandem mass spectrometric fragmentation pathways of small molecules using density functional calculations

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RATIONALE: Tandem mass spectrometry (MS/MS) dissociation pathways can vary markedly between compound classes and can result in challenging and time-consuming interpretation of the data. Compound, class and substructure specific fragmentation rules for protonated molecules require refinement to aid the structural elucidation process.
METHODS: The application of a predictive science approach using density functional theory (DFT) calculations has been investigated to estimate the abundances of first-generation product ions observed using an ion trap mass spectrometer. This has been achieved by application of Boltzmann population theory to electrospray ionisation (ESI)-MS and MS/MS data.
RESULTS: Tandem ESI-MS data for this preliminary study were used to investigate the internal stabilities of protonated species and their product ions. The calculated relative abundances of 11.3%, 96.5%, and 1.1% for the product ion (*m/z* 192) of three quinazoline structural isomers are compared with the experimental values of 16%, 90% and 0% observed in the first-generation product ion mass spectra.
CONCLUSIONS: Close correlation between calculated and experimental data has been demonstrated for these initial data. Applying this approach and establishing fragmentation rules, based on structure specific and common fragmentation behaviour, would improve and expedite the structural elucidation process. Copyright © 2013 John Wiley & Sons, Ltd.

For more than 30 years computational approaches have been used in mass spectrometry (MS) to aid the characterisation, understanding and prediction of the fragmentation behaviour of gas-phase ions.[1,2] The DENDRAL project was one of the first to focus on the problem of structural elucidation of unknown molecules using computational approaches.[3] At that time, the most common method of ionisation in mass spectrometry was electron ionisation (EI), the latter resulting in extensive investigations of the fragmentation behaviour of small molecules. Many databases, as well as structural elucidation matching software packages,[3,4] were developed. Improvements in computer technology enhanced the capabilities of structure predictive software for EI-MS approaches leading to projects such as CHEMICS and MASSIMO.[5–7] These two projects had a similar aim, i.e. to develop software that could predict mass spectral data for a given structure. The creation of larger databases,[8,9] with the incorporation of mass spectrometry rules into the predictive software, e.g. isotope pattern recognition, led to increased confidence for matching a chemical structure to the mass spectrum of an unknown compound. The continued expansion of these databases ensured the successful construction of many EI-MS libraries, such as the US National Institute of Standards and Technology library.[10,11]

More recently electrospray ionisation (ESI) low-energy fragmentations, e.g. collision-induced dissociation (CID) MS/MS analyses, have been implemented and library databases constructed, although these are not as comprehensive as standard EI-MS libraries. The dissociation mechanisms of these ESI-MS/MS spectra can be specific to an individual compound class, subclass or structurally similar scaffold. Consequently, the mass spectral interpretation of these data can be a difficult time-consuming process and this time variable is often dependent on the individual analyst.

General observation and tenets of gas-phase dissociation have been used and also integrated into these approaches to improve the data interpretation process, i.e. the even electron rule[12] or Stevenson's rule.[13] For example, collision-induced dissociations afford the formation of even electron product ions and even electron losses because even electron ions are more stable;[14] radical cations are rarely formed due to their high reactivity and instability.[15,16]

Many ESI-MS databases consider the even electron rule as the principal rule for the fragmentation behaviour of gas-phase ions. Since odd electron driven collision-induced dissociations have been observed in MS/MS data, peak

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identifications using methodologies that are based around these even electron rules can be missed.[17,18]

Fragmentation prediction software is continually developing; initially these programs were simple bond fragmenters based on fundamental mass spectrometry rules, such as the nitrogen rule and the even electron rule.[19] These suites were originally based on EI fragmentations but latterly improved to include even electron ionisation techniques, e.g. atmospheric pressure ionisation. However, the fragmentation behaviour of some structurally similar compounds can differ and structural elucidation becomes even more difficult if different mass analysers are employed.[20] Through implementation of compound-, class- and instrument-specific rules, garnered from library data, some software packages now use neural networks to learn new processes from the entered data. A limitation of these approaches is that currently available prediction software packages must contain reference data.[21]

Structurally similar compounds may dissociate to give common fragmentation patterns, common product ions and/or common losses. Therefore, the identification of the product ions would not necessarily improve the absolute identification of the compound since these ions may not be structure-specific.[22] Only through the identification of structure-specific product ions can the data interpretation process lead to the identification of a particular compound or class of compounds. Specific functional groups, sites of substitution and compound class can show distinctive fragmentations and therefore enhance the structural elucidation process.

Different theoretical approaches have been considered in an attempt to understand some specific fragmentation behaviours. These have mainly used thermodynamic approaches to the fragmentation behaviour of compounds while there has been much less activity using kinetic theories.[23–26] The transition state calculations or molecular dynamics (involving kinetic theories) applied to gas-phase ion reactions often challenge the generally held views of the mechanisms and pathways of the fragmentation behaviour.[27,28] The use of commercially available computational software, e.g. Gaussian,[29] Jaguar[30] or Spartan,[31] has become more widespread outside the computational chemistry community because their simpler user-friendly interface requires less specialist computing knowledge.[32,33] *Ab initio* quantum chemistry calculations, based on density functional theory (DFT), have often been used to help understand the specific fragmentation behaviour of small molecules.[1] Well-optimised simulations should improve the knowledge of atom interactions on a molecular level and help the understanding of dissociation patterns of small molecule gas-phase ions. Most of the *in silico* studies of small molecule calculations are performed using the B3LYP hybrid exchange-correlation functional,[1] which is commercially available in most DFT software packages.[32,33] For example, Vessecchi's approach, including the transition state species, presents a coherent explanation of specific fragmentation mechanisms.[26] The integration of mass spectrometry with DFT calculations should facilitate the interpretation of the fragmentation behaviour of small molecules.[32] DFT-MS/MS approaches discussing the influence of the change of a bond length during the protonation process have been published.[34,35] One of the conclusions was that the actual site of protonation influences the change or changes in the lengths of bonds that may be several bonds distant from the site of protonation. These bonds may cleave during the fragmentation process,[34] in agreement with observations that led to the creation of the bond-activation reinforcement rule (BAR rule) reviewed in detail by Alcami *et al*.[1] The change in bond length is related to the charge density of the two atoms involved in the protonation process. The BAR rule focuses on the differences in electronegativity of the two atoms[1] and excludes the rationale of measuring the dissociation energy of bonds that may also be relevant, although are not necessarily bond-length dependent.[36] The bond energies were implemented in a new prediction model based on *ab initio* approaches called Fragment iDentificator (FiD).[37] This involves the analysis of possible single-step and multi-step fragmentation pathways as well as hydrogen rearrangement reactions.

A disadvantage of any of the prediction software packages is the compromise of computational cost and speed of analysis versus higher prediction score and accuracy of result.[38] For most DFT models, the compromise between simulation time and accuracy means that simulations are limited to 50 atoms, excluding hydrogen atoms; thus applications are mainly focused on small molecules and metabolites.[39,40] For example, a simulation for a larger molecule (> 50 atoms) would demand a longer calculation time, improved computational power and therefore increased cost. The time required for a particular calculation will depend on the choice of hardware, software and the selected model. Thus, the computational time of conventional DFT calculations is scaled to N^3, where N is the number of orbitals in the calculation, and therefore is dependent on the number of atoms and the quality of a basis set; i.e. calculations for larger molecules with more atoms mean that more orbitals are taken into account, resulting in longer calculation times.[39,40]

Current computational approaches to develop prediction software for small molecule fragmentation are not focused on a deep understanding of the fragmentation behaviour of gas-phase ions. Establishing a set of rules, based on structure-specific and common fragmentation behaviour of subclasses of compounds, would simplify the structural elucidation process. By selecting the optimum features from pre-existing models and supplementing this knowledge with rules for specific compound classes could enhance MS/MS prediction software without the need to upload reference data. However, the use of different mass analysers and/or different ion activation methods can lead to method-specific fragmentation behaviour. Whilst there may also be some generic, cross-platform rules to consider, any fragmentation rules established will need to include mass-analyser-specific dissociations. Finally, this improved prediction software could aid, but not substitute for, the analyst in structural elucidation studies. Hill and Mortishire-Smith's approach is a good example of prediction software.[41] The aim of their model, called EPIC (Elucidation of Product Ion Connectivity), was focused on reducing the number of possible product ions by identifying the precursor ion using high-resolution mass spectrometry (HR-MS) and incorporating the accurate mass measurement (AMM) into the prediction software.

This work investigates the fragmentation behaviour of positional isomers of quinazolines using ESI-CID-MS/MS. The understanding of specific dissociation patterns, to aid structural elucidation, has been related to DFT calculations

using a purely thermochemical approach. Understanding and predicting the fragmentation behaviour of small molecules based on the DFT calculations have been supplemented by combining additional knowledge gained from hydrogen/deuterium exchange (HDX) and AMM experiments. This approach should improve the structural elucidation process of small molecules by understanding the energetics that drive gas-phase ion mechanisms and therefore aid prediction of MS/MS data and structural elucidation.

EXPERIMENTAL

Chemicals

The quinazolines, 7-benzyloxy-6-methoxyquinazolin-4-one (compound 1), 6-benzyloxy-7-methoxyquinazolin-4-one (compound 2) and 7-benzyloxy-8-methoxyquinazolin-4-one (compound 3), were supplied by AstraZeneca (Macclesfield, UK) (Fig. 1).

Stock solutions of 1 mg mL^{-1} were prepared in methanol (LC-MS grade, Fisher Scientific Ltd, Loughborough, UK) and stored in a fridge, from which 1 μg mL^{-1} solutions methanol/formic acid (0.1%) (Analytical reagent grade, Fisher Scientific Ltd) were prepared daily. The HDX experiments were performed using solutions prepared as above using deuterated methanol (MeOD-4, >99.5%) and deuterated formic acid (DCOOD, >99.0%) (Apollo Scientific Ltd, Stockport, UK).

Instrumentation

Positive ion electrospray product ion mass spectra were acquired using a LCQ Classic QIT (quadrupole ion trap) mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) operating with Xcalibur 1.2 software (Thermo Fisher Scientific). MeOH and MeOD sample solutions were directly infused at a constant flow rate of 3 μL min^{-1}. Nitrogen was used as the sheath and auxiliary gas, and helium as the collision gas. The source conditions were as follows: sheath gas flow rate 30 arbitrary units, auxiliary gas flow rate 1 arbitrary unit, spray voltage 3.7 kV, capillary temperature 200 °C, capillary voltage 31 V and tube lens offset voltage 5 V. Spectra were obtained with an isolation width of either 1 or 4 *m/z* units. The 1 *m/z* unit isolation width was applied to simplify the product ion spectra and observe the ion corresponding to the ^{12}C isotope only. The normalised collision energy was optimised for each compound and was varied from 20% to 50% for MS/MS experiments with respect to the WideBand activation being on or off. Each spectrum was the result of 20 averaged scans. The activation time and collision time were kept constant.

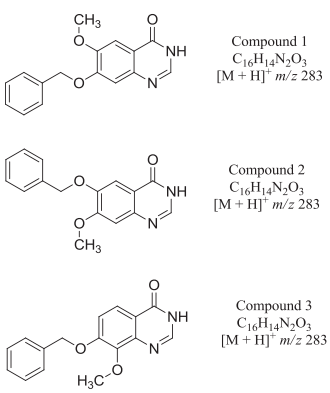

Figure 1. Structures of quinazoline positional isomers.

Accurate mass measurements were performed using an Apex III FTICR mass spectrometer with a 4.7 T actively shielded magnet with an external Apollo ESI ion source (Bruker Daltonics, Inc., Bremen, Germany). Sample solutions were directly infused at a constant eluent flow rate of 3 μL min^{-1}. The mass range of experiments was set between *m/z* 65 and *m/z* 650 and data were acquired using 512 K data points. Nitrogen was used as drying gas and argon as collision gas. Source parameters for positive ion ESI-MS were as follows: capillary voltage −4.5 kV, spray shield voltage −3.8 kV, capillary exit voltage 80 V, drying gas temperature 250 °C, skimmer 1 voltage 12 V, and skimmer 2 voltage 6 V. The ICR cell voltages were PV1 1.25 V, PV2 1.20 V and excite plate PL3 6 V. The values of corr sweep (PL4) and ion activation (PL8) attenuations, which are responsible for the isolation and fragmentation, respectively, of ions of selected *m/z* values, were varied for each MS/MS experiment to ensure that the peak isolation and fragmentation parameters were optimized for each specific compound. The first generation product ion spectra were acquired when the signal intensity for the protonated molecule was reduced to a minimum, to produce a spectrum with the highest abundance of product ions; thus allowing data to be compared with product ion spectra acquired on a QIT mass spectrometer. The ESI Fourier transform ion cyclotron resonance mass spectrometry (FTICR) MS/MS spectra were acquired using Xmass 7.0.8 (Bruker Daltonics, Inc.) and all data were processed using Data Analysis 3.4 (Bruker Daltonics, Inc.).

Theoretical calculations

The DFT calculations were performed using Spartan '02 (Wavefunction Inc., Irvine, CA, USA).[31] A validated method was used to calculate the protonation energies using single point energies, B3LYP exchange-correlation functional and the 6-31G* basis set on the energetically lowest geometry conformers optimised by the Merck molecular force fields (MMFF) approach.[38,39,42] The most favourable site of protonation, for each of the protonated molecules, may be indicated by analysis of the enthalpies of protonation (ΔH_{prot}).[25,43] However, the premise is that protonation of a molecule does not occur on one favoured atom, but is distributed across all possible sites of protonation.[33] Thus, a distribution of the protonated molecule species may be formed. The use of Boltzmann distribution theory (based upon changes of enthalpies of protonation only) was used to estimate the distribution of each species by population studies[40] (described in percentage values) and to identify the particular forms of protonated molecule (FPM, i.e. a species with a specific tautomeric site and specific site of protonation) that are responsible for a specific fragmentation behaviour. The temperature was varied between 0 K and ambient and the difference observed in the population studies was negligible (1–2%) between these two values.

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RESULTS AND DISCUSSION

Mass spectrometry

The comparison of first-generation product ion spectra of three positional isomers is presented in Fig. 2. The spectrum of protonated compound 1 (Fig. 2(a)) shows the formation of a product ion at *m/z* 91, $C_7H_7^+$ (data confirmed by AMM and presented in the Supporting Information, Tables S.I.1–S.I.3 – for each compound). However, product ions formed due to the loss of species such as MeOH or CO are also observed. Similarly, protonated compound 3 (Fig. 2(c)) fragments to form a base peak at *m/z* 91. However, there are fewer product ions formed in the higher mass range (*m/z* 160–283). The fragmentation behaviour of protonated compound 2 (Fig. 2(b)) does not show such similarities; *m/z* 91 is present but only at less than 3% abundance and the base peak (*m/z* 192) has been confirmed as a radical cation formed through the loss of the benzyl radical ($C_7H_7^{\bullet}$). This base peak at *m/z* 192 is also observed in the product ion spectrum of protonated compound 1, but at a lower relative abundance. Product ions due to the loss of smaller fragments from protonated compound 2 are not observed.

Computational approach

The probability studies discussed here are based on thermodynamic studies only and are applied in order to understand the formation of a specific product ion in three positional isomers, i.e. the radical cation, *m/z* 192. It is known that radicals are reactive species; however, they can be thermodynamically stable if charge re-localisation is possible. Therefore, specific product ions (in this case radical cations) can be used to help understand their formation in CID experiments and help to build rules of fragmentation.

The enthalpies of protonation for the formation of a precursor ion, *m/z* 283, and the product ion, *m/z* 192, for three positional isomers were calculated. The protonated molecule, as well as the product ion, can have more than one site of protonation and tautomeric form. Each quinazoline can have three possible tautomeric forms, as shown in Fig. 3 for compound 1. In addition, compounds 1, 2 and 3 each have five most probable sites of protonation, i.e. the heteroatoms, which equate to 15 possible forms of protonated molecule for each compound. Analogously, 15 possible forms of the product ion can be proposed.

Individual forms of the protonated molecule may dissociate to form specific, analogous forms of product ions. Correlation of the population studies of hypothetical product ions to the population of the forms of protonated molecules aids the understanding of the mechanism that drives the specific dissociations and can reduce the number of possible mechanistic pathways.

Quantification of the spread of theoretical species of protonated molecules was performed using Boltzmann

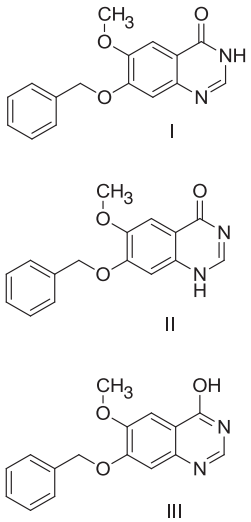


Figure 3. Structures of three tautomeric forms of compound 1.

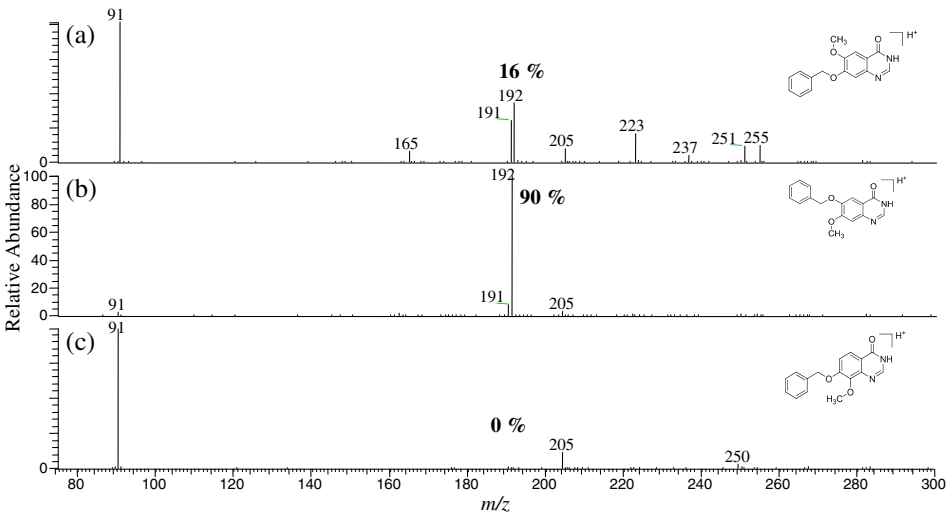


Figure 2. Positive ion electrospray ionization first generation product ion mass spectra of the protonated molecule (*m/z* 283): (a) compound 1, (b) compound 2 and (c) compound 3 (MeOH solutions); isolation width = 1 *m/z* unit. The percentage values represent relative, normalised, abundance values of *m/z* 192.

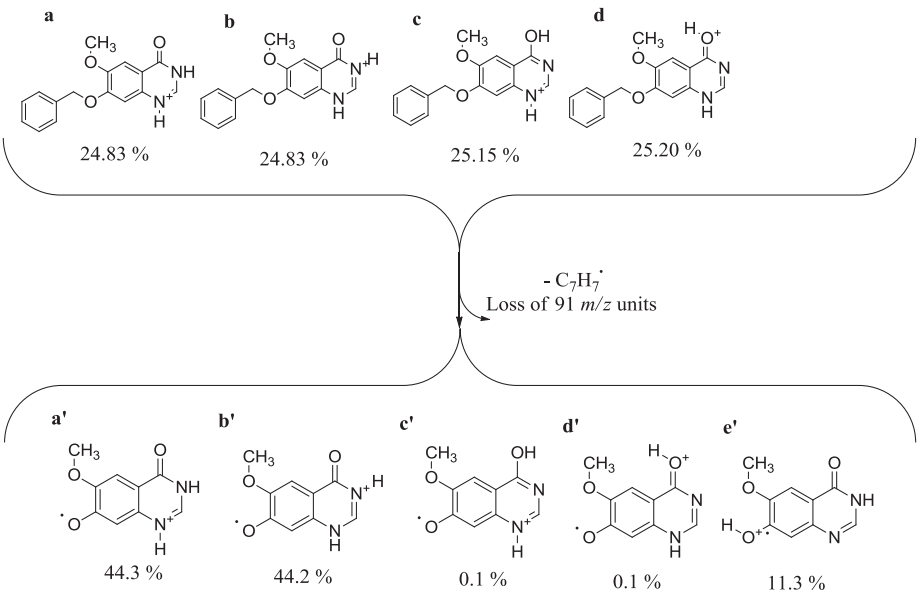
distribution theory. The ΔH_{prot} values of all 15 possible species were calculated and the most favoured forms, in normalised populations (percentages), are presented in Scheme 1. Compound 1 is the most thermodynamically stable of the three compounds and population studies indicate four forms of protonated molecule (a, b, c and d) and five forms of product ion at *m/z* 192 (a′, b′, c′, d′ and e′) (schemes for compounds 2 and 3 are available in the Supporting Information, Schemes S.I.1 and S.I.2). The four indicated forms of protonated molecule are estimated to be ca. 25% each. The first two forms of product ions, i.e. a′ and b′, have the highest probability of formation, estimated at ca. 44%. It is also observed that the c′ and d′ analogue forms of product ions are theoretically less favourable, ca. 0.1%, but notably one additional form of product ion, e′, has a thermodynamically probable formation of ca. 11.3%. The e′ form of product ion does not have an analogous form of protonated molecule (this would be e, in the upper row), and has also been indicated in the theoretical calculations of compounds 2 and 3. The probability of formation of the equivalent to the e′ form of product ion in compounds 2 and 3 has been calculated to be 96.5% and 1.1%, respectively (Supporting Information, Schemes S.I.1 and S.I.2).

The hypothetical analogue form of the protonated molecule would support the suggestion of a simple homolytic cleavage due to the loss of 91 *m/z* units. However, in a scheme such as this, the additional e′ form of the product ion must be formed from a, b, c or d of the thermodynamically indicated forms of the protonated molecule. Therefore, the mechanistic pathway resulting in the formation of the e′ form has to be more complex than just a simple odd electron dissociation. It must involve charge migration to the oxygen atom where the elimination of the benzyl radical has occurred. A movement of a charge to the cleaved bond seems to be thermodynamically favoured and agrees with experimental data. Depending on the possible mechanism of this process, a hydrogen atom that moves to a different site (of protonation) is known as a mobile proton.[44] It is interesting that one of the most stable and favourable forms of the radical cation species of the three positional isomers has been indicated as a protonated species with the charge on the radical oxygen atom.

The benzyloxy substituent of the three compounds is involved in two fragmentation mechanisms: (i) formation of *m/z* 91 ($C_7H_7^+$) and (ii) formation of a radical cation at *m/z* 192 ($[M+H–C_7H_7^{\bullet}]^{+\bullet}$). The dissociation of the protonated molecules of the three compounds described results in the formation of one or both of these product ions and is considered to be a structurally competitive process. The understanding of these two competitive mechanism pathways could aid the interpretation of the fragmentation behaviour and consequently improve the rule-based predictive approach.

The favourability of the formed product ions, i.e. their abundances in first-generation product ion spectra, can be correlated to thermodynamic stabilities indicated by Boltzmann distribution studies. As a result, thermodynamic studies of the e′ form of the product ion indicate relative abundances of 11.3%, 96.5% and 1.1%, which are in good correlation with the experimental values of 16%, 90% and 0% for *m/z* 192 in the first-generation product ion mass spectra of protonated compounds 1, 2 and 3, respectively. This confirms that the predictive DFT approach can provide a link to mass spectral data (Fig. 2).

It is significant that protonation on the oxygen atom leads to one of the most stable and favourable forms of product ion, which is counter-intuitive to the generally held belief that protonation in ESI occurs at the most basic site of a molecule. Further acceptance of the non-adherence to the even electron rule, with focus on the option of odd electron driven processes directing fragmentation, will improve the structural elucidation process. It is noteworthy that two concurrent odd electron losses result in an even electron product ion, which



Scheme 1. Schematic population distribution (% values) of most thermodynamically favourable forms of protonated molecule (top row) and forms of product ion (bottom row, *m/z* 192) of compound 1 indicated from heats of formation calculated by single point energies B3LYP/6-31G*.

itself may be a bi-radical. The latter observation has been noted in related structures (data not shown here) and will be discussed in a future publication.

CONCLUSIONS

The use of density functional theory (DFT) calculations together with mass spectrometry provides a better understanding of gas-phase ion mechanisms and can aid MS/MS data prediction and structural elucidation. The DFT calculations of enthalpies of protonation are a useful tool to understanding the specific CID fragmentation of small molecules. Population studies, in addition to HDX and AMM experiments, may readily determine and narrow the range of proposed mechanisms for CID-MS/MS dissociations. Structural elucidation studies may be further improved if density functional studies are applied to a larger range of compounds.

Modelling of the populations of different species of protonated molecules or formed product ions can help define specific mechanistic dissociation pathways. This is also aligned to the hypothesis that there is not one favoured site of protonation on the molecule and that the site with the highest proton affinity does not have to be the most crucial site for fragmentation. Density functional studies indicated that more than one form of protonated molecule and product ion can be formed with respect to the site of protonation and the proportion of the preferred forms can be quantified using population studies. Each of the thermodynamically favoured forms of protonated molecule may be involved in more than one mechanism; however, one form can dominate one fragmentation pathway. It was noted that odd electron species are energetically more stable when the protonation site is defined as the oxygen atom of the homolytically cleaved bond.

The approach described above highlights that DFT-MS/MS could play an important role in a predictive science approach for the interpretation of tandem MS data. The combination of common rule-based software with a DFT approach would efficiently advance the use of predictive science in mass spectrometry. In addition, HDX and AMM experiments are just as important in understanding the thermodynamic and kinetic properties of gas-phase ions. If both approaches are used, then rules could be defined and integrated into computational rule-based DFT software for small molecule structural elucidation. Expansion of the DFT approach could be applied to higher molecular weight compounds or faster simulations by the application of linear-scaling DFT codes, these approaches would then enable analysis of compounds of a thousand atoms or more.[45]

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article

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REFERENCES

[1] M. Alcamí, O. Mó, M. Yáñez. Computational chemistry: A useful (sometimes mandatory) tool in mass spectrometry studies. *Mass Spectrom. Rev.* **2001**, *20*, 195.
[2] L. Radom. Chemistry by computer: A theoretical approach to gas-phase ion chemistry. The 1990 Allan Maccoll Lecture. *Org. Mass Spectrom.* **1991**, *26*, 359.
[3] B.G. Buchanan, A.M. Duffield, A.V. Robertson, in *Mass Spectrometry: Techniques and Applications*, (Ed.: G.W. Milne), John Wiley, New York, **1971**.
[4] D.H. Smith, B.G. Buchanan, R.S. Engelmore, A.M. Duffield, A. Yeo, E.A. Feigenbaum, J. Lederberg, C. Djerassi. Applications of artificial intelligence for chemical inference. VIII. Approach to the computer interpretation of the high resolution mass spectra of complex molecules. Structure elucidation of estrogenic steroids. *J. Am. Chem. Soc.* **1972**, *94*, 5962.
[5] S. Sasaki, Y. Kudo. Structure elucidation system using structural information from multisources: CHEMICS. *J. Chem. Inf. Comput. Sci.* **1985**, *25*, 252.
[6] A.H. Lipkus, M.E. Munk. Automated classification of candidate structures for computer-assisted structure elucidation. *J. Chem. Inf. Comput. Sci.* **1988**, *28*, 9.
[7] J. Gasteiger, W. Hanebeck, K.P. Schulz. Prediction of mass spectra from structural information. *J. Chem. Inf. Comput. Sci.* **1992**, *32*, 264.
[8] K. Funatsu, N. Miyabayashi, S. Sasaki. Further development of structure generation in the automated structure elucidation system CHEMICS. *J. Chem. Inf. Comput. Sci.* **1988**, *28*, 18.
[9] B. Fan, H. Chen, M. Petitjean, A. Panaye, J.-P. Doucet, H. Xia, S. Yuan. New strategy of mass spectrum simulation based on reduced and concentrated knowledge databases. *Spectrosc. Lett.* **2005**, *38*, 145.
[10] F.W. McLafferty, D.B. Stauffer. Retrieval and interpretative computer programs for mass spectrometry. *J. Chem. Inf. Comput. Sci.* **1985**, *25*, 245.
[11] S.E. Stein, in *NIST Chemistry WebBook, NIST Standard Reference Database Number 69*, NIST Mass Spec Data Center, (Eds: P.J. Linstrom, W.G. Mallard). National Institute of Standards and Technology, Gaithersburg, MD, 20899.
[12] F.W. McLafferty. *Mass Spectrometry of Organic Ions*. Academic Press, New York, **1963**.
[13] D.P. Stevenson. Ionization and dissociation by electronic impact. The ionization potentials and energies of formation of sec.-propyl and tert.-butyl radicals. Some limitations on the method. *Discuss. Faraday Soc.* **1951**, *10*, 35.
[14] J. Delfosse, W. Bleakney. Dissociation of propane, propylene and allene by electron impact. *Phys. Rev.* **1939**, *56*, 256.
[15] F.W. McLafferty. *Interpretation of Mass Spectra: An Introduction*. W.A. Benjamin, New York, **1967**.
[16] M.E. Rose, R.A.W. Johnstone. *Mass Spectrometry for Chemists and Biochemists*. Cambridge University Press, London, **1982**.
[17] Y. Cai, Z. Mo, N.S. Rannulu, B. Guan, S. Kannupal, B.C. Gibb, R.B. Cole. Characterization of an exception to the 'even-electron rule' upon low-energy collision induced decomposition in negative ion electrospray tandem mass spectrometry. *J. Mass Spectrom.* **2010**, *45*, 235.
[18] W. Wagner, H. Heimbach, K. Levsen. Gaseous odd- and even-electron ions. *Int. J. Mass Spectrom. Ion Phys.* **1980**, *36*, 125.
[19] R. Mistrik. Determination of molecular structures using tandem mass spectrometry. Number 7197402, in United States Patents, **2007**.
[20] A. Galezowska. PhD thesis, University of Southampton, Southampton, UK, **2011**.
[21] S. Neumann, S. Bocker. Computational mass spectrometry for metabolomics: Identification of metabolites and small molecules. *Anal. Bioanal. Chem.* **2010**, *398*, 2779.

[22] M.L. Bandu, K.R. Watkins, M.L. Bretthauer, C.A. Moore, H. Desaire. Prediction of MS/MS data. 1. A focus on pharmaceuticals containing carboxylic acids. *Anal. Chem.* **2004**, *76*, 1746.
[23] A.K.Y. Lam, R.A.J. O'Hair. Isomer differentiation via collision-induced dissociation: The case of protonated α-, β2- and β3-phenylalanines and their derivatives. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 1779.
[24] S.J. Blanksby, S. Kato, V. Bierbaum, G.B. Ellison. Fragmentations of deprotonated alkyl hydroperoxides ROO upon collisional activation: a combined experimental and computational study. *Aust. J. Chem.* **2003**, *56*, 459.
[25] R. Vessecchi, S.E. Galembeck. Evaluation of the enthalpy of formation, proton affinity, and gas-phase basicity of gamma-butyrolactone and 2-pyrrolidinone by isodesmic reactions. *J. Phys. Chem. A* **2008**, *112*, 4060.
[26] R. Vessecchi, P. Nascimento, J.N.C. Lopes, N.P. Lopes. Fragmentation studies of synthetic 2-acylamino-1,4-naphthoquinones by electrospray ionization mass spectrometry. *J. Mass Spectrom.* **2006**, *41*, 1219.
[27] J.M. Mercero, J.M. Matxain, X. Lopez, D.M. York, A. Largo, L.A. Eriksson, J.M. Ugalde. Theoretical methods that help understanding the structure and reactivity of gas phase ions. *Int. J. Mass Spectrom.* **2005**, *240*, 37.
[28] L.J. Haupert, J.C. Poutsma, P.G. Wenthold. The Curtin–Hammett Principle in mass spectrometry. *Acc. Chem. Res.* **2009**, *42*, 1480.
[29] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery, T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, A. Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople. *Gaussian*. Wallingford, USA, **2003**.
[30] *Jaguar, Version 7.8*. Schrödinger, LLC, New York, **2011**.
[31] *Spartan'02*. Wavefunction, Inc., Irvine, USA.
[32] R. Vessecchi, C.A. Carollo, J. N.C. Lopes, A.E.M. Crotti, N.P. Lopes, S.E. Galembeck. Gas-phase dissociation of 1,4-naphthoquinone derivative anions by electrospray ionization tandem mass spectrometry. *J. Mass Spectrom.* **2009**, *44*, 1224.
[33] P. Wright, A. Alex, T. Nyaruwata, T. Parsons, F. Pullen. Using density functional theory to rationalise the mass spectral fragmentation of maraviroc and its metabolites. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 1025.
[34] A. Alex, S. Harvey, T. Parsons, F.S. Pullen, P. Wright, J.-A. Riley. Can density functional theory (DFT) be used as an aid to a deeper understanding of tandem mass spectrometric fragmentation pathways? *Rapid Commun. Mass Spectrom.* **2009**, *23*, 2619.
[35] K. Klagkou. PhD thesis, University of Southampton, Southampton, UK, **2003**.
[36] Y.-R. Luo. *Comprehensive Handbook of Chemical Bond Energies*. CRC Press Taylor & Francis Group, Boca Raton, USA, **2007**.
[37] M. Heinonen, A. Rantanen, T. Mielikainen, J. Kokkonen, J. Kiuru, R.A. Ketola, J. Rousu. FiD: a software for *ab initio* structural identification of product ions from tandem mass spectrometric data. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 3043.
[38] C.J. Cramer. *Essentials of Computational Chemistry. Theories and Models*, (2nd edn.), John Wiley, Chichester, UK, **2004**.
[39] W.J. Hehre. *A Guide to Molecular Mechanics and Quantum Chemical Calculations*. Wavefunction, Inc., Irvine, USA, **2003**.
[40] D.C. Young. *Computational Chemistry: A Practical Guide for Applying Techniques to Real-World Problems*. John Wiley, New York, **2001**.
[41] A.W. Hill, R.J. Mortishire-Smith. Automated assignment of high-resolution collisionally activated dissociation mass spectra using a systematic bond disconnection approach. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 3111.
[42] K. Gundertofte, T. Liljefors, P.-O. Norrby, I. Pettersson. A comparison of conformational energies calculated by several molecular mechanics methods. *J. Comput. Chem.* **1996**, *17*, 429.
[43] J.-F. Gal, P.-C. Maria, E.D. Raczyńska. Thermochemical aspects of proton transfer in the gas phase. *J. Mass Spectrom.* **2001**, *36*, 699.
[44] A.R. Dongré, J.L. Jones, Á. Somogyi, V.H. Wysocki. Influence of peptide composition, gas-phase basicity, and chemical modification on fragmentation efficiency: evidence for the mobile proton model. *J. Am. Chem. Soc.* **1996**, *118*, 8365.
[45] C.-K. Skylaris, P.D. Haynes, A.A. Mostofi, M.C. Payne. Introducing ONETEP: Linear-scaling density functional simulations on parallel computers. *J. Chem. Phys.* **2005**, *122*, 084119.

