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Correlation of Ecom₅₀ values between mass spectrometers: effect of collision cell RF voltage on calculated survival yield

Dennis W. Hill¹, Clive L. Baveghems¹, Daniel R. Albaugh², Tzipporah M. Kormos^{1,3}, Steven Lai⁴, Hank K. Ng¹, and David F. Grant^{1,*}

¹Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, 69 North Eagleville Road, Storrs, Connecticut 06269, USA

²Boehringer Ingelheim Pharmaceuticals, Inc, 900 Ridgebury Rd., Ridgefield, CT 06877, USA

⁴Waters Corporation, 100 Cummings Center, Beverly, MA 01915, USA

Abstract

RATIONALE—The determination of the center-of-mass energy at which 50% of a precursor ion decomposes (Ecom₅₀) during collision-induced dissociation (CID) is dependent on the chemical structure of the ion as well as the physical and electrical characteristics of the collision cell. The current study was designed to identify variables influencing Ecom₅₀ values measured on four different mass spectrometers.

METHODS—Fifteen test compounds were protonated using +ve electrospray ionization and the resulting ions were fragmented across a range of collision energies by CID. Survival yield versus collision energy curves were then used to calculate Ecom₅₀ values for each of these [M+H]⁺ ions on four different mass spectrometers. In addition, the relative recovery of the [M+H]⁺ ions of eight compounds ranging in molecular weight from 46 to 854 Da were determined at collision cell RF voltages ranging from 0 to 600 V.

RESULTS—Ecom₅₀ values determined on the four instruments were highly correlated (r² values ranged from 0.953 to 0.992). Although these overall correlations were high, we found different maximum ion recoveries depending on collision cell RF voltage. High mass ions had greater recovery at higher collision cell RF voltages, whereas low mass ions had greater recovery at lower collision cell RF voltages as well as a broader range of ion recoveries.

CONCLUSIONS—Ecom₅₀ values measured on four different instruments correlated surprisingly well given the differences in electrical and physical characteristics of the collision cells. However, our results suggest caution when comparing Ecom₅₀ values or CID spectra between instruments without correcting for the effects of RF voltage on ion transfer efficiency.

Introduction

In a mass spectrometric analysis the survival yield (SY) is the fraction of precursor ion remaining at some specified time following decomposition energy activation^[1]. In collision induced dissociation (CID) analysis the specified time is that required for the precursor and product ions to reach the detector. SY analysis in mass spectrometers has served as a useful method to understand energy distribution and gas-phase reaction mechanisms that occur during CID experiments.^[1–6] Using SY analysis, the total ion current can be quantified as the sum of surviving precursor ions and newly formed product ions and the sequence of

*Correspondence to: David F. Grant, University of Connecticut, 69 N Eagleville Rd, Storrs, CT 06269, USA., david.grant@uconn.edu.

³Current address: U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, Research Triangle Park, NC 27711, USA

appearance of product ions can be related to bond types and relative bond strengths. Thus, precursor ion survival and the abundance and structures of the product ions produced in CID are clearly dependent on chemical structure.

In CID reactions, kinetic energy is transferred to a precursor ion through its collision with neutral gas molecules or atoms.^[7] The amount of energy absorbed by the accelerated ion is dependent on the velocity of the ion, the mass of the ion and the mass of the collision gas. The theoretical amount of energy that can be absorbed in such a reaction is called the center-of-mass energy (E_{com}) and is given by equation 1

$$E_{com} = E_k M_g / (M_x + M_g) \quad (1)$$

where, E_k is the kinetic energy (in eV) of the sample ion, M_g is the molecular weight of the collision gas and M_x is the monoisotopic mass of the ion.^[7-9]

The energy absorbed by the accelerated ion is distributed among the ion's oscillators in the form of quanta. Quanta of energy can be distributed among the oscillators in a number of ways depending on the amount of energy absorbed. Each time the oscillators vibrate, the energy becomes redistributed. When an oscillator accumulates the number of quanta equal to its critical energy it will dissociate. If the total amount of energy absorbed by an ion equals the critical energy of its lowest energy oscillator, it will only dissociate if all of the quanta are distributed to that oscillator. Since there are a number of other oscillators in the ion, the probability of this occurring is very low. However, since there is a redistribution of the quanta of energy when the oscillators vibrate, the longer the ion retains this energy, the greater the probability that the critical oscillator will become filled with quanta of energy and dissociate. The probability per unit time of an oscillator becoming filled with the critical quanta of energy is the dissociation constant of the reaction. The greater the amount of absorbed energy above the critical energy, the greater the probability of filling the critical oscillator in a shorter time period.^[10-12] Ions with higher dissociation constants have a greater probability of fragmenting before they are transported to the detector and thus they result in a greater abundance of the decomposition product represented in the CID spectrum.

We have recently shown^[13] that the calculated center-of-mass energy required to decompose 50% of the precursor ion (E_{com50}) is dependent on the structure of the parent molecule and that this relationship can be used to predict the E_{com50} of a precursor ion. This method has the potential to aid in the identification of unknown compounds by comparing their experimentally determined E_{com50} values with those predicted for a set of candidate structures. However, because the composition and quantity of product ions detected in a CID spectrum is dependent on the configuration of the collision cell and its relationship to the detector^[9], it was of interest to determine whether E_{com50} values for $[M + H]^+$ ions determined on one instrument could be correlated with those determined on instruments of different configurations. In the present study we compared E_{com50} values for a set of protonated reference compounds on four different instruments having various types of collision cells. In addition, we evaluated the effects of collision cell RF voltage settings on E_{com50} values. Our results suggest that E_{com50} values are fairly consistent among instruments; however, the RF voltage of the collision cell can dramatically affect the results. This was especially evident when the product ions were significantly lower in mass than their precursor ions.

EXPERIMENTAL

Materials

p-Chlorobenzylpyridinium, p-cyanobenzylpyridinium, p-fluorobenzylpyridinium, p-methoxypyridinium, p-methylbenzylpyridinium, p-nitrobenzylpyridinium and p-tertbutylbenzylpyridinium were provided by Valerie Gabelica and Edwin De Pauw (Laboratoire de Spectrometrie de Masse, Universite de Liege, Liege, Belgium) as 1 μ M solutions in 1:1 (v/v) acetonitrile:water. These were used without further purification. 4-Dimethylaminopyridine, glycine, 8-hydroxyquinoline, benzimidazole, benzoxazole, formamide, 8-hydroxyquinoline, hypoxanthine, indole, nicotinamide, strychnine, and paclitaxel were purchased from Sigma-Aldrich (St. Louis, MO, USA). Reserpine was from Ciba-Geigy (St. Louis, MO, USA), and terbutaline sulfate from United States Pharmacopeia (Rockville, MD, USA). HPLC grade acetonitrile (99.9%) was purchased from Fisher Scientific (Pittsburgh, PA, USA) and heptafluorobutyric acid was purchased from Thermo Scientific (Rockford, IL, USA). Reagent grade water was generated in-house on a Barnstead system (Thermo Scientific, Asheville, NC, USA).

Instrumentation

All compounds were analyzed in positive ion mode using an electrospray ionization (ESI) source at a flow rate of 5 μ L/minute on Qtof-2 (Waters Corporation, Beverly, MA, USA), Qstar Elite (Applied Biosystems, Concord, Ontario, Canada) and Synapt G2 (Waters Corporation) mass spectrometers, and at 10 μ L/minute on a LTQ-Orbitrap XL (Thermo Scientific, Waltham, MA, USA) mass spectrometer. A ZsprayTM ionization source (Waters Corporation) was used on the Qtof-2 and the Synapt G2 mass spectrometers. The source temperature was 120° C. The desolvation gas temperature was 120° C on the Qtof-2 and 100° C on the Synapt G2. Nitrogen was used as the nebulizer gas at an inlet pressure of 100 psi and the cone gas at a flow rate of 50 L/h on both instruments. The desolvation gas was nitrogen at a flow rate of 450 L/h on the Qtof-2 and 400 L/h on the Synapt G2. The capillary voltage was set at 3.2 kV on both instruments and the cone voltage was 20 V on the Qtof-2 and 50 V on the Synapt G2. Solutions of test compounds were delivered to the ionization sources using a Harvard Apparatus syringe pump (Holliston, MA, USA). CID spectra of protonated test compounds were generated in a hexapole collision cell in the Qtof-2 and in a T-wave trap collision cell on the Synapt G2. Ions exiting the Qtof-2 collision cell passed through a short hexapole transfer cell prior to entering the TOF detector. Argon was used as the collision gas on the Qtof-2 (10 psi head pressure, $5-8 \times 10^{-3}$ mbar manufacture's estimated gas cell pressure) and Synapt G2 (2.3×10^{-3} mbar cell pressure) mass spectrometers. Masslynx version 4.0 software (Waters Corporation) was used to process mass spectral data obtained on the Qtof-2 and Synapt G2 mass spectrometers.

Solutions of test compounds were delivered to a TurboIonTM (Applied Biosystems) electrospray source on the Qstar Elite using a built-in syringe pump. Nitrogen was used as the source and curtain gas at flow rates of 0.89 L/min and 0.92 L/min, respectively. The capillary voltage on the Qstar Elite source was 5.5 kV and the declustering potential 1 setting was 80 (arbitrary units) for all compounds except p-methoxybenzylbenzylpyridinium for which a value of 45 was used to reduce in-source collision-induced-dissociation. The declustering potential 2 setting was 15 (arbitrary units) and the focusing potential setting was 260 (arbitrary units). Nitrogen was used as the collision gas at an inlet pressure setting of 10 (arbitrary units) in the hexapole collision cell. Analyst QS version 2 software (Applied Biosystems) was used to process mass spectral data obtained on the Qstar Elite mass spectrometer.

Test samples were infused into an Ion Max atmospheric pressure ionization source (Thermo Scientific, Waltham, MA, USA) on the LTQ-Orbitrap XL using a builtin syringe pump. The source voltage was 4 kV, the tube lens voltage was 90 V, the capillary voltage was 35 V and the capillary temperature was 275°C. The higher energy collision dissociation cell with nitrogen as the collision gas was used on the Orbitrap XL. Mass spectral data were analyzed using Xcalibur version 2.1 software (Thermo Scientific, Waltham, MA).

The Qtof-2 and Qstar Elite systems utilized a quadrupole mass filter in series with a hexapole collision cell. The Synapt G2 mass spectrometer used a quadrupole mass filter in series with a T-wave collision cell. The Orbitrap XL system utilized a linear ion trap mass filter in series with an octapole collision cell.

The collision cell on the Qtof-2 mass spectrometer, which was used to study the effects of collision cell RF voltage on ion transfer efficiency, was composed of six stainless steel rods 160.5 mm in length arranged in a circle with a 9 mm inscribed diameter and 2 mm entrance and exit slits. The transfer cell located between the collision cell and the TOF analyzer consisted of six stainless steel rods 48 mm in length arranged in a circle with a 9 mm inscribed diameter. The entrance slit to the transfer cell was the exit slit of the collision cell and there was no exit slit on the transfer cell.

Determination of Ecom₅₀ values of protonated test compounds on different instruments

Test solutions of benzimidazole, benzoxazole, p-chlorobenzylpyridinium, p-cyanobenzylpyridinium, 4-dimethylaminopyridine, p-fluorobenzylpyridinium, hypoxanthine, 8-hydroxyquinoline, indole, p-methoxybenzylpyridinium, p-methyl benzylpyridinium, nicotinamide, p-nitrobenzylpyridinium, strychnine, and p-tertbutylbenzylpyridinium were prepared at appropriate concentrations in 1:1 aqueous 0.01% (v/v) trifluoroacetic acid:acetonitrile. The test compounds were analyzed on the Qtof-2, Qstar Elite, Synapt G2, and Orbitrap XL mass spectrometers either individually or in mixtures of compounds that differed in molecular weight. Sample compounds were infused into the respective ESI sources and the resulting [M+H]⁺ ions were individually isolated and subjected to CID in the respective collisions cells. CID spectra were obtained for the [M+H]⁺ ion of each test compound using a range of collision energies that resulted in 80% SY to 20% SY for each precursor ion. The SY values at each collision energy were calculated from equation 2

$$SY = I_M / (I_M + \sum I_F) \quad (2)$$

where I_M is the peak intensity of the [M+H]⁺ precursor ion and $\sum I_F$ is the sum of the peak intensities of the product ions. The CE₅₀ values were calculated from the SY values as previously described^[14]. The Ecom₅₀ values were determined using the protonated compound's CE₅₀ value, its protonated monoisotopic molecular weight and the reagent gas molecular weight as described in Equation 1 where the CE₅₀ = E_k.

Effect of RF voltage on relative ion transfer efficiency in collision/transfer cell

Solutions of eight compounds ranging in molecular weight from 46 to 854 Da were prepared at various concentration in 0.01% trifluoroacetic acid in water:acetonitrile (1:1). Solution A contained benzimidazole (0.4 µg/mL; protonated mass [PM] 119 Da), 8-hydroxyquinoline (0.4 µg/mL; PM 146 Da) and terbutaline (0.4 µg/mL ; PM 226 Da), solution B contained a mixture of strychnine (0.5 µg/mL; PM 335 Da), formamide (10 µg/mL; PM 46 Da), reserpine (2.0 µg/mL; PM 609 Da) and glycine (10 µg/mL; PM 76 Da) and solution C contained 10 µg/mL paclitaxel (PM 854 Da). Each solution was infused in triplicate into the

ESI source of the Qtof-2 mass spectrometer. The resulting $[M+H]^+$ ions were isolated and accelerated at 5 eV into the collision cell containing argon. The CID spectra of each precursor ion were determined with collision and transfer cell RF voltages set at 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, 420, 480, 540 or 600 V. The RF values of the collision and transfer cells were held constant at 963 KHz. The relative ion transfer efficiency was determined as the ratio of the precursor ion peak area intensity obtained at each RF voltage to the precursor ion peak area intensity at the RF voltage that gave the greatest response.

Effect of collision energy on ion transfer efficiency in collision/transfer cell

To determine the effect of collision energy on the collision RF voltage relative ion transfer efficiency curves, a 0.4 $\mu\text{g/mL}$ solution of benzimidazole was analyzed on the Qtof-2 mass spectrometer as described in the previous section at different collision/transfer cell RF voltages using collision energies of 18.4, 25.3 and 32.2 eV. The relative ion efficiency versus RF voltage curves at these collision energies and the one previously determined at 5 eV were compared to determine if collision energy had an effect on the relative ion transfer efficiency.

The effect of collision energy on relative ion transfer efficiency was studied by performing CID analyses on the protonated benzimidazole (m/z 119.1) using the Qtof-2 mass spectrometer with helium as the collision gas. The collision energies ranged from 1 to 67 eV at 2 eV intervals. If collision energy has no effect on ion transfer efficiency, the intensity of the $[M+H]^+$ ion should be constant at all collision energies since insufficient internal energy is available for fragmentation upon collision with He atoms at any of the collision energies used.

Results and Discussion

Figure 1 shows the linear correlation of the $E_{\text{com}50}$ values obtained on the Qstar Elite, Synapt G2 and the Orbitrap XL mass spectrometers with those determined on the Qtof-2 mass spectrometer. $E_{\text{com}50}$ values were obtained for the $[M+H]^+$ ions of all fifteen test compounds on the Qtof-2 and the Qstar Elite instruments. The linear regression comparison of the $E_{\text{com}50}$ values determined on these two instruments gave a small positive intercept and a slope slightly less than 1 (Fig. 1A) with a correlation coefficient of 0.9818. Using parameters similar to those used on the Qtof-2 for the $E_{\text{com}50}$ analysis on the Synapt G2, the apparent SY values for protonated benzimidazole, benzoxazole and nicotinamide did not fall below 0.5. The linear regression comparison of the $E_{\text{com}50}$ values of the other twelve protonated compounds with those obtained on the Qtof-2 gave a slightly negative y-intercept, a slope very close to 1 and a good correlation coefficient (Fig. 1B). On the Orbitrap XL mass spectrometer, protonated p-methylpyridinium and p-methoxypyridinium did not decompose to below 50% at any collision energy tested. The $E_{\text{com}50}$ correlation of the other thirteen protonated test compounds with those determined on the Qtof-2 mass spectrometer gave a slightly positive linear regression y-intercept, a slope less than 1 and a somewhat lower correlation coefficient than those observed between the Qtof-2 and the Qstar Elite or Synapt G2 (Fig. 1C). The usefulness of these correlation equations is the ability to correct $E_{\text{com}50}$ values obtained on one instrument to those obtained on a reference instrument that has been used to create a chemical structure- $E_{\text{com}50}$ relationship model to predict $E_{\text{com}50}$ values. Given the dependence of fragmentation on the physical configuration and electrical parameters of the collision cell, it is somewhat surprising that the $E_{\text{com}50}$ values correlated between different instruments as well as they did. However, there were some $[M+H]^+$ ions that demonstrated greater differences in relative $E_{\text{com}50}$ values between instruments than others and $E_{\text{com}50}$ values could not be obtained on some instruments under the parameters used in the initial analysis.

There are a number of reasons why the apparent change in the rate of decomposition of an ion would be measured differently between mass spectrometers. Once an ion has absorbed a sufficient amount of energy to decompose it will not do so until the critical quanta of energy are distributed to the respective oscillators. The probability of this occurring is time dependent and it will not occur unless the activated ion has been resident in the collision cell for a sufficient amount of time. The amount of time for which an activated ion remains in the collision cell depends on its velocity in the collision cell and the length of the cell. The velocity of the ion is dependent on the kinetic energy applied to the ion and the m/z value of the ion. The length of the collision cell is a physical characteristic, which may be different in different mass spectrometers.

Regardless of the kinetic energy of a sample ion entering a collision cell, the probability of it decomposing before it reaches the end of the cell is very close to 0 if it does not collide with a collision gas atom or molecule. The probability of an ion colliding with a collision gas atom or molecule increases with the concentration of the gas in the collision cell, i.e. the collision gas pressure. Implicit in the interpretation of the E_{com50} as a structural characteristic is that every precursor ion entering the collision cell will collide with at least one collision gas molecule or atom. It has been shown that the SY of a group of precursor ions at a specific kinetic energy above the critical energy decreases with increased collision gas pressure in an asymptotic relationship^[15]; the maximum representing nearly all the precursor ions being involved in at least one collision. The collision gas pressures should therefore be near the asymptotic maximum for correlation of E_{com50} values between instruments. In the current study it was difficult to establish the same collision gas pressure between instruments, because only one of the instruments used had the capability to directly measure this parameter.

Since the calculation of center of mass energy compensates for the mass of the collision gas, the E_{com50} values for the same protonated compound determined using Ar or N₂ as the collision gas should be the same. Supplementary Figure S1 (Supplementary Material) shows the linear regression comparison of the E_{com50} values determined for a set of nine protonated compounds analyzed using Ar and N₂ as the collision gas. The values of the linear regression slope and the correlation coefficient are very close to one, indicating that the E_{com50} values determined using these two collision gases are correlative.

One of the assumptions in the calculation of the E_{com50} is that the sum of the intensities of the product and precursor ions that survive passage through the collision cell is proportional to the intensity of the precursor ion entering the collision cell, regardless of the collision energy. This is usually considered approximately true in published studies that use calculated SY values^[3, 4, 14, 16, 17] and requires the ion transfer efficiency through the collision cell (and ion optics prior to the detector) to be the same for the precursor ion and all product ions. In addition, the molar response of the detector must be the same for all ions. The ion guide collision cells used in this study were maintained at a constant RF and moderate RF voltage during ion collision and transport through the cell. Most ions of different m/z value pass through a collision cell under these conditions. However, theoretical models of ion trajectories in multipole ion guides^[18] and experimental observations^[19] have shown that the transfer efficiencies of ions of different m/z values through collision cells vary with the magnitude of the constant RF and RF voltage applied to the cell. Figure 2 shows the intensity of $[M+H]^+$ ions of different m/z values relative to their maximum intensity at different RF voltages and constant RF in the Qtof-2 collision cell. It is observed that higher voltages enhance the recovery of higher mass ions and hinder the recovery of lower mass ions, with the voltage range of maximum recovery varying with the mass of the ion.

Ecom₅₀ values for the [M+H]⁺ ions of benzimidazole, benzoxazole and nicotinamide were not obtained on the Synapt G2 mass spectrometer when the routine operating voltage of the collision cell was 300 V because the apparent SY values of the precursor ions did not drop below 0.5 with increasing collision energy. However, when the collision cell RF voltage was reduced to 50 V, the apparent SY values of these protonated compounds approached 0 once sufficient collision energy had been applied. An example of this effect for protonated benzimidazole analyzed on the Synapt G2 is shown in Fig. 3. The lower response of the low mass product ions at the higher collision cell voltage results in a lower estimate of the initial precursor ion entering the collision cell, which causes the calculated SY to be greater than its actual value. A similar response is probably the cause of the inability to calculate Ecom₅₀ values for protonated p- methylpyridinium and p-methoxypyridinium on the Orbitrap XL mass spectrometer.

If the ion transfer efficiencies of different mass ions are known at specific collision cell RF voltages, one may theoretically correct the product ion intensities to a common recovery. Figure 4 shows the collision cell ion transfer efficiencies relative to the maximum intensity of ions at different *m/z* values using a 5 eV collision energy when the collision cell RF voltages were 90 V, 180 V, and 300 V, respectively, in the Qtof-2 mass spectrometer. These types of relationships may be used to correct the ion intensities of precursor and product ions to their theoretical maximum values. High relative transfer efficiencies are obtained for all but the lowest mass ions at RF voltages of 180 V and 300 V. However, it is probable that low mass ions of significant true abundance will not be detected at these RF voltages. For Ecom₅₀ determinations, it might be advantageous to sacrifice some ion recovery at higher masses in order to accurately detect the contribution of low mass ions to the total ion current of the spectrum. This would provide a more accurate estimation of the Ecom₅₀ value for protonated compounds that generate high abundant low mass product ions. For the Qtof-2 mass spectrometer an RF voltage of 90 V resulted in a relative ion transfer efficiency greater than 0.4 for all *m/z* values > 45. The Ecom₅₀ values for protonated benzimidazole determined on the Qtof-2 mass spectrometer using RF voltages of 90 V, 180 V, and 300 V were 5.82 eV, 6.32 eV, and 7.65 eV, respectively. These values were 5.92 eV, 5.94 eV, and 5.79 eV when the precursor and product ion intensities were corrected to maximum ion transfer efficiencies using linear interpolation of the respective curves in Fig. 4. Thus, in addition to correcting for Ecom₅₀ differences between RF voltages, this approach allows CID spectra collected at different RF voltages to be compared and it might be useful for standardizing CID spectra collected on different instruments. For example, Figs 5A–C show the CID spectra of protonated benzimidazole collected at a collision energy of 29 eV and cell RF voltages of 90, 180, and 300 V, respectively. The linear regression correlations between the spectra in Figs 5A and 5B, 5A and 5C, and 5B and 5C were 0.4215, 0.0236, and 0.6882, respectively. Figures 5D–F show the same spectra after the ion intensities were corrected to maximum ion transfer efficiencies using the appropriate curves in Fig. 4. The correlation coefficients between the spectra in Figs 5D and 5E, 5D and 5F, and 5E and 5F were 0.8237, 0.8947, and 0.9838, respectively, showing a convergence of spectral profiles in the intensity corrected spectra.

The ion transfer efficiency for the [M+H]⁺ ion of each compound shown in Fig. 4 was normalized to the maximum recovery of each respective ion at 5 eV. These corrections would be valid for 5 eV CID spectra if the maximum ion transfer efficiency for each ion was the same. However, it is not clear whether the ion transfer efficiency of a specific *m/z* ion at a given RF voltage is the same at different collision energies.

Figure 6 shows the change in relative ion transfer efficiency for protonated benzimidazole at different RF voltages at collision energies of 5.0, 18.4, 25.3, and 32.2 eV. These collision energies represent 100%, 80%, 50% and 20% SY at a collision cell voltage of 300 V. The

data suggest similar relative ion transfer efficiencies at all collision energies tested up to RF voltages of approximately 180 V. However, above 180 V the relative ion transfer efficiency at 5 eV is greater than at the other three collision energies. This might suggest an increase in precursor ion scatter in the collision cell as the internal energy increases above the critical limit of the lowest energy oscillator. However, we are limited in the interpretation of this data since we do not know the absolute efficiency of ion transfer in this collision cell.

If the ion transfer efficiency of precursor ions remains reasonably constant across collision energies, the SY values (and hence the E_{com50} values) calculated using the ratio of the precursor ion intensity during fragmentation to the precursor ion intensity without fragmentation may be more accurate. Figure 7 shows the change in the benzimidazole precursor ion relative intensity as a function of collision energy using He as the collision gas. The apparent recovery of the precursor ion increased with an increase in kinetic energy up to 13 eV. However, although the precursor ion did not decompose when using He as the collision gas (i.e. no detectable product ions were observed), the precursor ion signal steadily decreased to near 0 at 65 eV. The cause of this decrease in ion transport efficiency with increasing collision energy is not known. However, it could be due to increased ion scattering within the collision cell or decreased sampling efficiency in the TOF analyzer as ion velocities increase. Although the ion transfer efficiency appears to change with collision energy (as shown in Fig. 7), a valid correction might be feasible for precursor and product ions within a spectral profile at any specific collision energy. If the sums of the product and precursor ions are corrected to the same efficiency, the ratio of the corrected precursor ion intensity and the sum of the corrected precursor and product ion intensities should provide an accurate estimate of the true SY. These results suggest that E_{com50} values and CID spectra should be corrected for RF voltage and collision gas differences between instruments in order to make valid comparisons. However, theoretical calculations by Knyazev and Stein^[15, 20] indicate that the ion transfer efficiencies of product ions are lower than those of precursor ions. This suggests that a different set of ion transfer – RF voltage relationships would need to be developed for the correction of product ion intensities.

Conclusion

E_{com50} values determined on four different mass spectrometers were reasonably well correlated. Where differences existed, a relationship between collision cell RF voltage and maximum relative ion transport efficiency was used to obtain a corrected estimate of E_{com50} values. The use of corrected product and precursor ion abundances should provide E_{com50} values more closely related to the critical energy required for precursor ion fragmentation. In addition, the use of ion transfer efficiency corrected CID spectra might allow standardization across instrument platforms and thus facilitate comparison among CID spectra databases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Comparison of Ecom₅₀ values determined on a Qstar Elite (A), Synapt G2 (B), and Orbitrap XL (C) to those determined on the Qtof-2 mass spectrometer. Ecom₅₀ values for protonated benzimidazole, benzoxazole and nicotinamide are not included on graph B and Ecom₅₀ values of protonated p-methylpyridinium and p-methoxypyridinium are not included on graph C because these values could not be determined on the Synapt G2 and Orbitrap XL, respectively, under the parameters used in this study.

Figure 2.
Effect of collision cell RF voltage on relative ion transfer efficiency of ions of different masses. (n=3)

Figure 3.
Comparison of protonated benzimidazole SY curves at collision cell RF voltages of 50 V and 300 V on the Synapt G2.

Figure 4. Relationships of collision cell relative ion transfer efficiencies at collision cell RF voltages of 90 V (A), 180 V (B) and 300 V (C). (n=3)

Figure 5.

Uncorrected CID spectra of protonated benzimidazole measured at 29 eV and RF voltages of 90 V (A), 180 V (B), and 300 V (C) compared with these spectra (D, E, & F, respectively) corrected to maximum ion transfer efficiency.

Figure 6.
Effect of collision energy on collision cell relative ion transfer efficiency of protonated benzimidazole (m/z 119) at different collision cell RF voltages. (n=3)

Figure 7.
Effect of collision energy on collision cell relative ion transfer efficiency of protonated benzimidazole (m/z 119) using 180 V RF voltage and He as collision gas.