Research Article

Rapid Communications in Mass Spectrometry

Received: 30 November 2011 Revised: 10 February 2012 Accepted: 10 February 2012 Published online in Wiley Online Library

Rapid Commun. Mass Spectrom. **2012**, *26*, 1014–1022
(wileyonlinelibrary.com) DOI: 10.1002/rcm.6197

Spectral accuracy of a new hybrid quadrupole time-of-flight mass spectrometer: application to ranking small molecule elemental compositions

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RATIONALE: Determining the elemental compositions of unknown molecules is an important goal of analytical chemistry. The isotope pattern revealed by a mass spectrometer provides valuable information regarding the elemental composition of a molecule. In order to employ spectral accuracy considerations for elemental composition determination, it is important to know how faithfully a mass spectrometer can record the isotope pattern and to understand the magnitude of the errors of the relative isotopic abundances.

METHODS: Twenty-four small molecule drugs and two natural products representing a diverse range of elemental compositions and ranging in molecular weight from 236 to 1663 Da were measured on a new hybrid orthogonal acceleration quadrupole time-of-flight (Q-TOF) mass spectrometer by flow infusion analysis. The similarity between the observed profile isotope pattern and the theoretical isotope pattern, denoted spectral accuracy, was calculated using a computational algorithm in the program MassWorks.

RESULTS: The spectral accuracy for all compounds averaged better than 98%. When using spectral accuracy to rank elemental compositions with the elemental constraints ($C_{1–100}H_{0–200}N_{0–50}O_{0–50}F_{0–5}S_{0–5}Cl_{0–5}Br_{0–5}$) further restricted by empirical rules and a mass tolerance ≤5 parts-per-million, the correct formula was ranked first over 80% of the time. In contrast, when using mass accuracy for ranking, only two compounds (8%) were ranked first. For quinidine and troglitazone, the initial spectral accuracy measurements were lower than expected and further analysis indicated that minor, structurally related components were present.

CONCLUSIONS: Our work has determined the magnitude of spectral accuracy that can be expected on a new Q-TOF mass spectrometer. In addition, we demonstrate the utility of spectral accuracy measurements both for ranking elemental compositions and also for obtaining insight into the chemical nature of the analyte that might otherwise be overlooked.

Using mass spectrometry for elemental composition (EC) determination is an important application of analytical chemistry in diverse scientific fields including forensics,[1] toxicology,[2] environmental chemistry,[3] petrochemicals,[4] metabolomics,[5] and pharmaceuticals.[6] For molecules up to ~500 Da containing the elements $(CH)_{no\ limit}N_{0–5}O_{0–10}S_{0–3}$, a theoretical study reported that a mass accuracy of 0.1 mDa would give a unique EC.[7] Such mass accuracies are typically only obtained on Fourier transform ion cyclotron resonance (FTICR) mass spectrometers capable of resolving powers of $m/\Delta m$ (full width at half maximum (FWHM)) >400 000. For peptides with masses up to 700–800 Da, a theoretical study argued that a mass accuracy of ±1 ppm would give rise to a unique EC[8] in part because not all monoisotopic masses are possible for peptides. Small molecules have a greater variety of elements to consider and for this reason are more challenging than peptides. For low molecular weight analytes (<200 Da) high accuracy mass measurements (<1 ppm) may give a single EC. However, as the mass of the analyte increases, the number of feasible ECs increases exponentially and another theoretical study demonstrated that for metabolites from 0–500 Da with C, H, N, S, O, P, and potentially F, Cl, Br and Si as elements, even a mass accuracy < ±1 ppm is not sufficient to arrive at a unique EC when searching metabolite databases.[9] In such situations, adding constraints to the type and number of elements considered can reduce the number of ECs although with true unknowns this poses the risk of missing the correct EC.

Due to the limitations of mass accuracy alone, approaches have been developed to use other readily obtainable mass spectrometric information for EC determination. Making high mass accuracy measurements of both the protonated molecule and product ions following collision-induced dissociation allows the elimination of ECs inconsistent with observed product ions and neutral losses. When applied to 113 environmental chemicals with masses <410 Da using MS data collected on a quadrupole time-of-flight (Q-TOF) mass spectrometer, a unique EC was obtained for 65% of the compounds measured.[10] Recently, a software program called Multi-stage Elemental Formula has been developed which uses as input MS^n spectra from the mass spectral tree for a compound of interest.[11] Applying this program to 12 spectra for metabolites with masses from 150 to 450 Da acquired on an Orbitrap mass spectrometer at 30 000 resolving power (RP) provided a unique EC when considering

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the data acquired in MS^n experiments (n = 2–5) and it was also shown that when acquiring higher order spectra (MS^n), greater mass error was tolerated.

Isotopic peak ratios (i.e. isotope abundances) are a valuable source of information for EC determination as the natural abundances of isotopes of elements such as S, Br and Cl can give rise to informative isotopic clusters.[12] Including isotopic peak ratios can further limit the number of possible ECs for a given mass and should thus be considered, especially as the mass of the analyte increases.[9] Various workers have demonstrated the utility of including isotope abundances in addition to accurate mass of (1) precursor ion[1,13,14] and (2) precursor, product ions and neutral losses[15–17] for EC determination for a variety of analytes and matrices. Several reports have examined the accuracy of isotopic abundance measurements. In one study, 137 commercially available compounds with masses in the range of 75–810 Da were analyzed on an LTQ/Orbitrap mass spectrometer and the experimentally measured relative isotopic abundance (RIA) of [A+1]/A was compared with the theoretical one.[18] Approximately 40% of compounds had RIA errors between 0 and 3% with the remaining compounds equally distributed from 3–5%, 5–10%, 10–20% and >20%, respectively. Although another study that compared the quality of isotopic abundance measurements on an Orbitrap-Velos mass spectrometer at 100 000 RP and an LTQ/FT Ultra between 100 000 and 750 000 RP reported that these measurements were strongly dependent on the signal-to-noise ratio of the protonated molecule under investigation, these measurements did increase the number of single EC assignments by >3-fold compared with measuring accurate mass alone.[19]

Spectral accuracy (SA%), or spectral error (SE% = 100 – SA%), describes the goodness-of-fit between an observed and a theoretical isotope profile.[20] The commercially available software program MassWorks uses the algorithm self-Calibrated Line-shape Isotope Profile Search (sCLIPS) to calculate spectral accuracy. Briefly, this approach involves self calibrating the experimentally observed mass spectral peak shape to give a mathematically defined mass spectral profile spectrum which is then compared with an MS profile spectrum for a given EC calculated with the same mass spectral peak shape. The self-calibration algorithm uses the isotopically pure monoisotopic peak (e.g. the calibration range indicated in the Experimental section) of any ion as the model line-shape for calibration, which is then applied to the entire ion isotope pattern (e.g. the profile mass range indicated in the Experimental section). Unlike mass accuracy, a higher SA% implies a better match for a given EC. Spectral accuracy was evaluated on an Orbitrap-XL mass spectrometer for ten natural products with masses between 639 and 1663 Da at five RP settings. At a RP setting of 30 000, SA% ranged from 96.2 to 98.4% and for thiostrepton (1663 Da) the correct EC was within the top seven hits out of 1900 ECs, consistent with the elemental and 2 ppm mass accuracy constraints.[21] Instrument manufacturers have also developed algorithms to quantitatively score the match of a measured isotope pattern such as Bruker's Sigma-Fit® and Water's i-FIT®. The algorithm SIRIUS, which stands for Sum formula Identification by Ranking Isotope patterns Using mass Spectrometry, similarly determines ECs based solely on the exact mass and isotope distribution of the analyte under investigation which, when applied to spectra of organic compounds (100–900 Da) obtained on a Q-TOF mass spectrometer, correctly identified >90% of the metabolites as a top hit.[22]

With the growing recognition of the importance of using isotope abundance considerations for EC determination, it is important to know the fidelity of a mass spectrometer with regards to not only mass accuracy of the monoisotopic peak, but also the relative abundances of the isotopic peaks (A+1, A+2, etc.), i.e. the spectral accuracy. Thus, the aim of the present work was to evaluate spectral accuracy on a new oaQ-TOF mass spectrometer. To this end, a diverse range of elemental compositions was obtained consisting of 24 commercially available small molecule drugs ranging in mass from 236 to 704 Da, and two natural products with masses of 639 and 1663 Da (Table 1). Flow infusion analysis (FIA) was performed, the mass accuracy and SA% were determined, and the resulting ECs were ranked based on their SA%.

EXPERIMENTAL

Chemicals

Tenidap and citalopram were purchased from Tocris Bioscience (Bristol, UK), prochloperazine and trifluoperazine were purchased from MP Biomedicals (Solon, OH, USA) and moxidectin was purchased from Fluka (Stenheim, Switzerland). Troglitazone was purchased from Calbiochem (Gibbstown, NJ, USA). All other compounds analyzed in

Table 1. Molecular formulae and exact masses of the molecules investigated in the study

Group I	Formula	Exact Mass
Carbamazepine	$C_{15}H_{12}N_2O$	236.0944
Trimeterene	$C_{12}H_{11}N_7$	253.1070
Ketoprofen	$C_{16}H_{14}O_3$	254.0937
Propranolol	$C_{16}H_{21}NO_2$	259.1567
Metoprolol	$C_{15}H_{25}NO_3$	267.1829
Amitriptyline	$C_{20}H_{23}N$	277.1825
Imipramine	$C_{19}H_{24}N_2$	280.1934
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	295.0161
Group II		
Tenidap	$C_{14}H_9ClN_2O_3S$	320.0017
Quinidine	$C_{20}H_{24}N_2O_2$	324.1832
Citalopram	$C_{20}H_{21}FN_2O$	324.1632
Omeprazole	$C_{17}H_{19}N_3O_3S$	345.1142
Meloxicam	$C_{14}H_{13}N_3O_4S_2$	351.0342
Oxybutynin	$C_{22}H_{31}NO_3$	357.2298
Prochloperazine	$C_{20}H_{24}ClN_3S$	373.1374
Prazosin	$C_{19}H_{21}N_5O_4$	383.1588
Group III		
Trifluoperazine	$C_{21}H_{24}F_3N_3S$	407.1638
Ziprasidone	$C_{21}H_{21}ClN_4OS$	412.1119
Troglitazone	$C_{24}H_{27}NO_5S$	441.1604
Verapamil	$C_{27}H_{38}N_2O_4$	454.2826
Nicardipine	$C_{26}H_{29}N_3O_6$	479.2051
OH-taurosporine	$C_{28}H_{26}N_4O_4$	482.1949
Ketoconazole	$C_{26}H_{28}Cl_2N_4O_4$	530.1482
Moxidectin	$C_{37}H_{53}NO_8$	639.3766
Itraconazole	$C_{35}H_{38}Cl_2N_8O_4$	704.2388
Thiostrepton	$C_{72}H_{85}N_{19}O_{18}S_5$	1663.4918

the study were purchased from Sigma-Aldrich (St. Louis, MO, USA) as were di-butylamine acetate (DBAA), dimethyl isopropanolamine (DMIPA) and dimethyl sulfoxide (DMSO). Formic acid (>99%) was purchased from Acros Organics (Geel, Belgium) and HPLC grade acetonitrile was purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA). Water used was prepared from a MilliQ water system (Billerica, MA, USA).

FIA by electrospray ionization (ESI) on a Q-TOF mass spectrometer

Stock solutions (1 mM) for all standard compounds prepared in DMSO were diluted in water to 1–20 μM for positive or negative mode mass spectrometric analysis. This typically produced maximum ion abundances of the order of one to five million ions/scan although, for data processing, spectra with ion abundances of one hundred thousand ions/scan were averaged. A tripleTOF 5600 hybrid Q-TOF mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with a Shimadzu UFLC system (Kyoto, Japan), consisting of a CBM-20A control module, an SIL-30 AC autosampler, a DGU-20A5 degasser, and two LC-30 AD pumps, was used for FIA. The mass spectrometer was operated in the positive ion mode with a spray voltage of 5 kV or in the negative ion mode with a spray voltage of −4.5 kV. Ion source gas 1, ion source gas 2 and curtain gas (N_2) were set at pressures of 45, 50, 30 psi, respectively, and the source temperature was maintained at 600 °C. Samples (10 μL) were introduced into the mass spectrometer via the UFLC system by flow injection (no column) under isocratic conditions (50% A) at a flow rate of 0.3 mL/min. In positive ion mode, the mobile phase consisted of 0.1% formic acid in water (A) and 0.1 % formic acid in acetonitrile (B). In negative ion mode, the mobile phase consisted of 5 mM DBAA in water (A) and acetonitrile with 0.2% DMIPA (B).

Full scan mass spectra were acquired over the range of *m/z* 100–800 (except for thiostrepton, where the scan range was from *m/z* 900–1700) with an accumulation time of 0.1 s. A data-dependent experiment collecting three product ion spectra (70 ms each) was also included. The total acquisition time was 1.5 min per sample and all four time-to-digital converter channels were used for detection. Samples were injected on three separate days in randomized order. The tripleTOF instrument was calibrated by an integrated calibration delivery system (AB Sciex) using the manufacturer's positive and negative calibration solutions, with an injection flow rate of 200–300 μL/min. These calibrant ions were introduced via an orthogonal atmospheric pressure chemical ionization source together with mobile phase flow from the UFLC system. For sample analysis, calibration was performed every six samples. Mass spectral data acquisition and processing were performed using Analyst TF 1.5 (AB Sciex) and Peak View 1.1.1.2 software (AB Sciex).

SA% was calculated by MassWorks software (version 3.0.0, Cerno Bioscience, Danbury, CT, USA) using sCLIPS, which is a formula determination tool that first performs peak shape calibration and then matches the calibrated experimental isotope pattern against possible theoretical ones. The mass spectra were first converted into text files (.txt) and loaded sequentially into MassWorks for processing. The calculated ECs were ranked by decreasing SA% with the chemical elemental constraints ($C_{1–100}H_{0–200}N_{0–50}O_{0–50}F_{0–5}S_{0–5}Cl_{0–5}Br_{0–5}$) further restricted by enabling empirical rules[23] and a mass tolerance of 5 ppm, except for thiostrepton in which the elements Cl and Br were excluded. The profile mass range was chosen to include the monoisotopic peak and all other isotope peaks, e.g. –0.5 to 3.5 *m/z* units (relative to monoisotopic mass) depending on the compound, and the calibration range was ±0.2 *m/z* units from the monoisotopic peak. Searching was limited to even-electron ions. The standard deviations (SDs) reported in Table 2 were calculated over the three replicate analyses.

RESULTS

The results for the compounds analyzed will be described in three groups depending on the mass of the compound: group I <300 Da; group II >300 Da and <400 Da; group III >400 Da. When appropriate, specific compounds will be discussed in greater detail. SA%, SE%, mass error and resolving power (m/Δm, FWHM) for the compounds in each group are presented in Table 2.

Group I: compounds <300 Da

There were eight compounds in group I. All except ketoprofen were analyzed using (+) ESI. SA% values ranged from 96.77 ± 0.41 for diclofenac to 99.15 ± 0.03 for trimeterene, based on triplicate measurements corresponding to SE% between 3.23 and 0.85, respectively. The mass errors were less than 3 ppm for all compounds and averaged 1.59 ± 0.24 ppm. The distribution of SE and mass errors in mDa are illustrated in Fig. 1. The average resolving power (m/Δm) for these eight compounds was 29 082. All compounds in group I were ranked first based on having the highest SA% of the possible ECs consistent with the search parameters (Table 3). In contrast, if mass accuracy was used for ranking, only metoprolol and imipramine were ranked first with other compounds in this group ranging from 2 to 15. The difference in SA% between rank one and two averaged 2.66% for the compounds in group I.

Group II: compounds >300 Da and <400 Da

There were eight compounds in group II. All except tenidap were analyzed using (+) ESI. SA% values ranged from 98.25 ± 0.25 for tenidap to 99.13 ± 0.04 for citalopram based on triplicate measurements, corresponding to SE% between 1.75 and 0.87, respectively. The mass errors were less than 2.5 ppm for all compounds and averaged 1.48 ± 0.28 ppm. The average resolving power (m/Δm) for these eight compounds was 31 285. All compounds in group II were ranked first, based on having the highest SA% of the possible ECs consistent with the search parameters (Table 3). In contrast, if mass accuracy was used for ranking, no correct ECs were ranked first in this group. The difference in SA% between the top two ranks averaged 1.5% for the compounds in group II.

Quinidine

The initial results for quinidine gave a SA% of 86.57 ± 0.26, which was significantly different from that for the other compounds in this group. Upon closer evaluation of the

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Table 2. Spectral accuracy, spectral error, mass error and resolving power for compounds analyzed

Group	Compound	SA% ± SD	SE (%)	ppm	m/Δm
I	Carbamazepine	98.66 ± 0.03	1.33 ± 0.03	0.82	29 104
	Trimeterene	99.15 ± 0.03	0.85 ± 0.03	1.94	27 666
	Ketoprofen	97.49 ± 0.04	2.51 ± 0.04	2.89	30 097
	Propranolol	98.55 ± 0.22	1.45 ± 0.22	1.61	27 745
	Metoprolol	98.88 ± 0.20	1.12 ± 0.20	1.11	28 176
	Amitriptyline	97.81 ± 0.20	2.19 ± 0.20	2.07	29 736
	Imipramine	98.35 ± 0.30	1.65 ± 0.30	0.98	30 289
	Diclofenac	96.77 ± 0.41	3.23 ± 0.41	1.27	29 842
	Average	98.21 ± 0.59	1.79 ± 0.59	1.59	29 082
II	Tenidap	98.25 ± 0.25	1.75 ± 0.25	1.63	31 742
	Quinidine[a]	86.57 ± 0.26	13.43 ± 0.26	1.74	31 555
	Quinidine[b]	98.85 ± 0.05	1.15 ± 0.05	1.74	31 555
	Citalopram	99.13 ± 0.04	0.87 ± 0.04	2.44	29 700
	Omeprazole	99.09 ± 0.11	0.91 ± 0.11	0.23	30 454
	Meloxicam	99.10 ± 0.13	0.90 ± 0.13	2.20	31 281
	Oxybutynin	98.98 ± 0.08	1.02 ± 0.08	1.90	32 011
	Prochloperazine	98.82 ± 0.01	1.18 ± 0.01	0.60	31 332
	Prazosin	99.07 ± 0.06	0.93 ± 0.06	1.06	31 936
	Average	98.91 ± 0.29	1.09 ± 0.29	1.50	31 285
III	Trifluoperazine	99.27 ± 0.09	0.73 ± 0.09	0.83	32 329
	Ziprasidone	96.61 ± 0.92	3.39 ± 0.92	0.34	32 129
	Troglitazone[a]	86.97 ± 3.66	13.03 ± 3.66	1.48	30 925
	Troglitazone[b]	97.13 ± 0.10	2.87 ± 0.10	1.48	30 925
	Verapamil	98.99 ± 0.09	1.01 ± 0.09	2.06	31 651
	Nicardipine	99.15 ± 0.03	0.85 ± 0.03	0.15	32 661
	7-Hydroxytaurosp	99.04 ± 0.19	0.96 ± 0.19	2.16	31 590
	Ketoconazole	98.53 ± 0.17	1.47 ± 0.17	1.35	33 089
	Moxidectin	98.09 ± 0.37	1.91 ± 0.37	0.88	37 974
	Itraconazole	98.19 ± 0.32	1.81 ± 0.32	0.73	34 390
	Average	98.33 ± 0.94	1.67 ± 0.94	1.15	32 766
	Thiostrepton[c]	97.86 ± 0.83	2.14 ± 0.83	0.68	39 416
	Thiostrepton[d]	95.77 ± 1.30	4.23 ± 1.30	1.08	39 416

[a]Excluded from average; [b]mixture search; [c]best case n = 3; [d]n = 9. SD = standard deviation.

spectrum, it appeared that the A+2 isotope peak was slightly higher than expected (Fig. 2). A subsequent sCLIPS search was performed which allowed for the possibility that quinidine and dihydroquinidine (+2 hydrogens) were present as a mixture. A mixture search fitted the observed isotope pattern with more than one elemental composition. SA% for quinidine in this mixture search improved by over 10% to 98.85 ± 0.05.

Group III: compounds >400 Da

There were ten compounds in group III, which comprised eight small molecules and two natural products. All except moxidectin were analyzed using (+) ESI. SA% values ranged from 96.61 ± 0.92 for ziprasodone to 99.27 ± 0.09 for trifluoperazine, based on triplicate measurements, corresponding to SE% between 3.39 and 0.73, respectively. The mass errors were less than 2.2 ppm for all the compounds and averaged 1.01 ± 0.24 ppm. The average resolving power (m/Δm) for these ten compounds was 33 875. In group III, all replicates of trifluoperazine, ziprasidone, verapamil, and nicardipine were ranked first, based on having the highest SA% of the possible ECs consistent with the search parameters. Except for thiostrepton, which is discussed below, all the other compounds in group III were not ranked first for all replicates although no compound ranked lower than 5th place. For example, itraconazole was ranked in place one, two or five for the three replicates. In contrast, if mass accuracy was used for ranking, only nicardipine and ziprasidone were ranked in the top ten, with the other compounds ranked between 15 and 266. Except for thiostrepton, the difference in SA% between the top two ranks was less than 1% and averaged 0.47% for the compounds in this group.

Troglitazone

Initial results for troglitazone revealed a SA% of 86.97 ± 3.66, which was significantly different from that for the other compounds in the group. Upon closer evaluation of the spectrum, it appeared that there were two peaks lower in mass by one and two hydrogens (Fig. 3). A subsequent sCLIPS search was performed which allowed for the possibility that troglitazone and oxidized troglitazones with

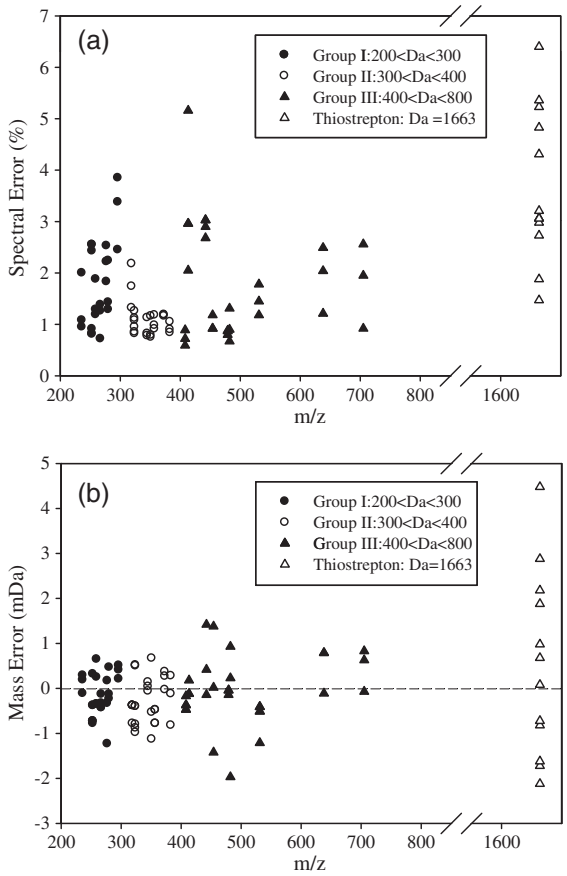

Figure 1. Mass errors in mDa (a) and spectral errors (SE%) (b) for compounds in groups I, II and III.

either 1 or 2 fewer hydrogens were present as a mixture. SA% for troglitazone in this mixture search improved by over 10% to 97.13 ± 0.10.

Thiostrepton

The highest mass compound analyzed, the natural product thiostrepton, posed some challenges when performing the sCLIPS calculations. When using our standard elemental and mass accuracy constraints, the number of possible ECs was found to be more than 146 000. Processing took several hours but was never completed before the program aborted. To overcome this problem, the elements Cl and Br were excluded and subsequent calculations generated about 7500 possible ECs. Although the first thiostrepton sample analyzed showed a very good SA% (98.53) and the correct EC tied for first place out of 7484 possible ECs, subsequent replicates had SA% values which were as much as 3% lower. Due to this variability, we carried out an additional six replicate analyses to get a better estimate of SA%. Again, of these six replicates only one sample was found to have a SA% of 98.12 and the variability remained larger than was observed for the other compounds (average SA% = 95.88 ± 1.28). The best ranking of thiostrepton was place one, five or ten for the three best SA% values obtained. However, for the other thiostrepton samples with lower SA%, rankings were in the hundreds (68–985) or even in the thousands (1035).

DISCUSSION

An early study by Ibanez and coworkers on a Q-TOF mass spectrometer noted that the accuracy of relative isotope abundance measurements was approximately 20% although the errors could be as high as 50% or less than 10%, depending on the ion intensity.[24] A more recent study by Abate and coworkers reported that the overall accuracies for the [A+1]/A and [A+2]/A isotope ratios for 344 species were 2.6 ± 2.5% and 2.1 ± 2.6%, respectively.[13] In our investigation, the overall fidelity for the measurement of protonated (or deprotonated) molecules was determined on a new oaQ-TOF mass spectrometer. To accomplish this objective, we obtained a set of small molecules and two natural products with a diverse set of ECs and used the sCLIPS algorithm to determine SA% of each protonated (or deprotonated) molecule. SA% (or SE% = 100% − SA%) is a measure of the overall goodness-of-fit between an observed and a theoretical protonated (or deprotonated) molecule for a given EC and thus considers not only the measured accurate mass of the monoisotopic peak, but also the relative abundances of the isotopic peaks (A+1, A+2, A+3, etc.). Based on the compounds that we measured, the results demonstrate that SA% averages better than 98% for an average SE% of 1.6%. The mass errors of the protonated (or deprotonated) molecules were also determined and it was found that on average the mass errors were better than 2.5 ppm. These findings indicate that the AB Sciex 5600 Q-TOF instrument meets the conditions suggested by Kind and Fiehn who predict that a mass spectrometer with both mass errors better than 3 ppm and isotope abundance accuracies of about 2% should outperform a mass spectrometer which has higher mass accuracy but which does not consider isotopic abundances for EC determination.[9] In contrast to the observations of Bristow and coworkers who found on a Bruker microQ-TOF mass spectrometer that simple isotope patterns (C, H, N, F, O) had higher ranking (i.e. better quality isotope pattern match),[25] our data indicates that SA% was not influenced by the presence of heteroatom(s) in the molecule. Specifically, the fifteen compounds in this study without heteroatoms had an average SA% of 98.68 ± 0.52% compared with the twelve compounds with heteroatoms (Cl, Br, or S) which had an average SA% of 98.16 ± 0.95% – not statistically different.

Limited elemental constraints and a 5 ppm mass tolerance were used for the EC determination. For compounds with masses between 236 and 412 Da, which included all compounds in groups I and II, the correct EC was ranked first out of as few as 20 (e.g. oxybutynin) to as many as 168 (e.g. ziprasidone) formulae. In contrast to SA%, when mass accuracy was used for ranking, only for two compounds in group I was the correct EC ranked first, as shown in Table 3. The significance of a large difference in SA% between the first and second hit compared with the standard deviation of the measurement is that it increases confidence in the top hit. For compounds in group I, the differences between the first and second rank were, as shown in Table 3, typically greater than 1% and as high as 4% (e.g. imipramine), which is about 5–10 times the standard deviation. For group II compounds, the difference between the first and second rank was still more than 1% but for compounds in group III this averaged much less than 1% and although the correct EC was ranked first at least in one analysis, at times the rank was two, three,

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Table 3. Ranking of compounds elemental composition based on spectral or mass error

Group	Compound	Rank (spectral error)	N[a]	SA% (1st - 2nd)	Formulae with SA ≥ 98%	Formulae with SA ≥ 95%	Rank (mass error)
I	Carbamazepine	1	8	3.71	1	1	2
	Trimeterene	1	10	1.02	2	4	7
	Ketoprofen	1	13	1.56	0	3	3
	Propranolol	1	9	3.53	1	1	3
	Metoprolol	1	5	2.43	1	3	1
	Amitriptyline	1	8	2.30	0	2	5
	Imipramine	1	7	4.14	1	2	1
	Diclofenac	1	38	2.57	0	1	15
	Average	1	12	2.66	1	2	5
II	Tenidap	1	83	1.64	1	6	22
	Quinidine	1	24	0.84	0	0	9
	Quinidine[b]	1	24	3.07	1	2	9
	Citalopram	1	29	2.53	1	3	8
	Omeprazole	1	48	2.49	1	5	2
	Meloxicam	1	89	1.67	2	7	33
	Oxybutynin	1	20	1.05	2	4	7
	Prochloperazine	1	66	1.85	1	2	2
	Prazosin	1	66	0.66	3	7	5
	Average	1	46	1.77	1	4	11
III	Trifluoperazine	1	91	0.58	2	7	16
	Ziprasidone	1	168	0.55	0	2	7
	Troglitazone	1,2 or 4	162	0.12	0	0	15
	Troglitazone[b]	1 or 2	684	0.32	0	9	15
	Verapamil	1	89	0.70	2	7	51
	Nicardipine	1	194	0.59	4	13	5
	OH-taurosporine	1	253	0.93	2	9	24
	Ketoconazole	1, 2 or 3	590	0.30	3	12	266
	Moxidectin	1 or 4	527	0.50	2	24	16
	Itraconazole	1, 3 or 5	2121	0.14	4	32	44
	Average	N/A	488	0.47	2	12	46
	Thiostrepton[c]	1, 5 or 10	7491	0.04	0–36	273–330	68
	Thiostrepton[d]	68–1035	7498	N/A	0–48	198–656	1452

[a]N = number of ECs obtained following the search; [b]mixture search; [c]best case n = 3; [d]n = 9.

four or even five. This indicates that slight variations in the spectral data quality can have an impact on SA% and be enough to alter the rank, especially when the number of possible ECs is in the hundreds or thousands (e.g. itraconazole, thiostrepton). Böcker and coworkers also divided the 86 compound spectra in their data set, acquired on a MicroQ-TOF from Bruker, into groups and showed a similar trend to ours with respect to correct ranking and mass.[22] For compounds with masses 200–300 Da or 300–400 Da, the correct EC was ranked first for every compound except one. However, of the ten compounds with mass 500–600 Da, only seven were ranked first and of three compounds with mass 800–900 Da, only two were ranked first.

Initially, SA% of both quinidine and troglitazone was determined to be approximately 87% and this led to further investigation of these two compounds since this was much lower than for the other compounds. Dihydroquinidine is the reduced form of quinidine that is present in various amounts in commercially available sources.[26] Thus, we next calculated SA% based on a mixture of quinidine and dihydroquinidine and this increased SA% to >98%. Moreover, our analysis indicated that the amount of dihydroquinidine was approximately 11%, consistent with literature reports.[27] Figure 2 shows the increased intensity of the A+2 peak of quinidine due to the contribution of the monoisotopic peak of dihydroquinidine. Troglitazone can undergo oxidative metabolism on the chromane system to form a quinone metabolite (loss of two hydrogen atoms). Electrochemical oxidation, which can occur during ESI, also readily generated this quinone.[28] Furthermore, generation of a radical with loss of a single hydrogen atom has also been reported.[29] Thus, we calculated SA% based on a mixture of troglitazone/oxidized troglitazone with loss of one and two hydrogen atoms and this increased SA% to >97%. Although initially overlooked due to their low abundance, a closer examination of protonated troglitazone revealed two peaks, 1 and 2 Da lower than the monoisotopic peak of troglitazone. Figure 3 shows that the change in the monoisotopic peak of troglitazone due to the presence of these other species significantly reduces SA% although their absolute intensity is low. In a previous investigation on an orbitrap in which both rifampicin and its quinone oxidation product were present

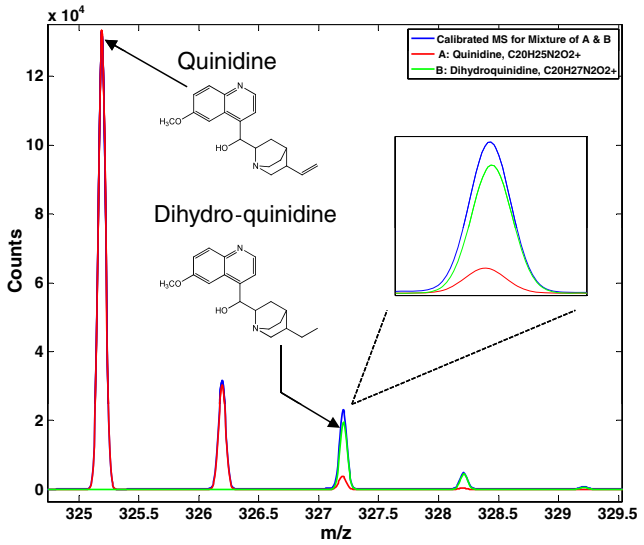

Figure 2. Spectrum of quinidine (red) with a small amount of dihydroquinidine (+2 *m/z* units, green) detected as a minor component. The observed spectrum (blue) is a summation of these two components. Dihydroquinidine increases the abundance of the A+2 isotope peak of quinidine as can be seen clearly in the insert. Note that the resolution of the spectrum was set to 0.7 *m/z* units (FWHM) for illustrative purposes.

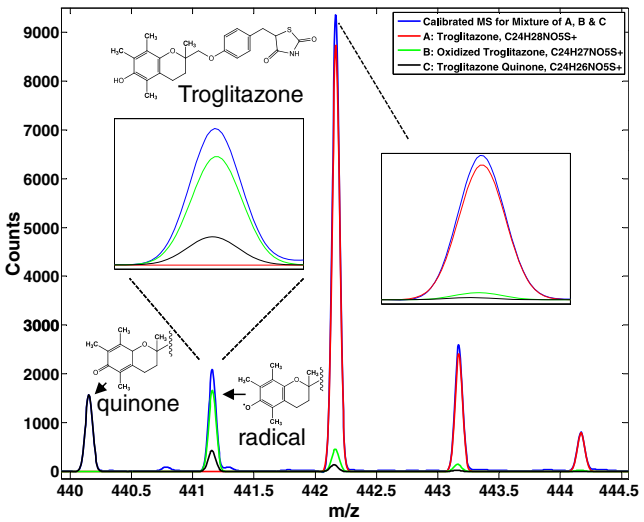

Figure 3. Spectrum of troglitazone (red) with smaller amounts of troglitazone quinone (−2 *m/z* units, black) and oxidized troglitazone (−1 *m/z* unit, green) detected as a minor components. The observed spectrum (blue) is a summation of these three components. The monoisotopic isotope peaks of oxidized troglitazone and troglitazone are increased slightly because of the presence of these components as can be seen clearly in the insert. Note that the resolution of the spectrum was set to 0.7 *m/z* units (FWHM) for illustrative purposes.

together in a commercially available source, SA% also improved when a mixture search was performed, although the improvement (~1%) was much smaller than the 10% improvements observed here.[21] These examples demonstrate that SA% determinations can point the investigator towards additional structural information and chemical insight into the analyte that might otherwise be easily overlooked.

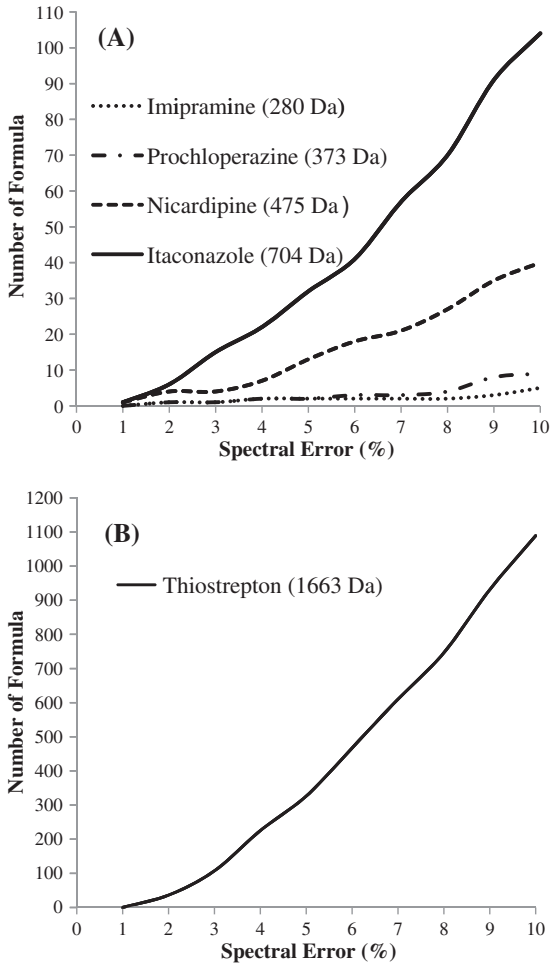

Figure 4. (a) Relationship between SE% and number of ECs for imipramine, prochloperazine and itraconazole as representative compounds of groups I, II and III, respectively. (b) Relationship between SE% and number of ECs for thiostrepton, which shows that when the SE% increases above approximately 3%, the number of ECs is in the hundreds.

In similar experiments to ours on an LTQ/Orbitrap-XL instrument, SA% values of ten natural products were determined at five different RP settings.[21] At a RP setting of 30 000, equivalent to the RP obtained on the present Q-TOF instrument, SE% values ranged from 1.62 to 3.8%. Fortunately, a direct comparison of SE% for two compounds is possible: moxidectin 2.11% vs. 1.9% and thiostrepton 3.1% vs. 3.7% on the Orbitrap and Q-TOF, respectively. However, at lower RP settings on the LTQ/Orbitrap-XL, the SE% values were better than on the Q-TOF: 1.58% for moxidectin at 15 000; 2.38% and 1.41% for thiostrepton at 15 000 and 7500, respectively. At higher RP settings (i.e. 60 000 and 100 000), SA% was shown to decrease on the orbitrap, especially with compounds, such as thiostrepton, possessing extensive fine structure. This was manifested as decreasing isotope abundances in the higher isotope peaks (i.e. A+3, A+4, A+5 ...). Erve and coworkers suggested that destructive interference between isotopic fine structure components that can occur in FTICR instruments accounts for these decreased isotopic abundances and hence lower SA%.[21] Although the Q-TOF would not be expected to

be subject to these effects, thiostrepton displayed a better SE% (3.59) on the orbitrap at 60 000 RP and was only worse than the Q-TOF at 100 000 RP (SE% = 5.64). It is not clear why the Q-TOF used in this study could not provide SA% values consistently of the order of 98% for thiostrepton.

The findings with thiostrepton best illustrate the importance of having a high SA% when the mass of the compound increases. With the best SE% values obtained, namely 1.47, 1.88 and 3.06, thiostrepton did rank among the top ten ECs (among >7400 ECs). However, for unknown reasons, this SE% was not consistently obtained and the greater SE% resulted in thiostrepton rankings in the hundreds or even thousands. Employing a lower mass tolerance of 2.7 ppm in the sCLIPS search could not improve the rankings enough to make them truly useful (data not shown). The relationship between the number of possible ECs and SE% is further illustrated in Fig. 4(a) using imipramine (group I), prochloperazine (group II), itraconazole (group III) and Fig. 4(b) with thiostrepton (group III) as examples. With groups I and II, significantly greater SEs (up to 10%) could be tolerated and still only give rise to a small number of ECs. However, as the mass increases, especially above 600 Da, having SE% <2 becomes extremely important both for ranking of and confidence in EC findings.

CONCLUSIONS

Determination of elemental composition (EC) for unknowns remains an analytical challenge and a strategy involving SA% determination on a mass spectrometer with high fidelity of isotope measurement capability (<3% SE), such as the AB Sciex 5600 Q-TOF, should be a powerful tool. For analytes with mass <400 Da, ranking based on SA% produced the top hit exclusively. Only with masses >400 Da did the rankings become more variable although, except for thiostrepton, the rank was always in the top ten. SA% determinations in our work were based on commercially available compounds and thus ion intensity was not an issue as the full isotope profile was readily observed. Although we did not systematically investigate the influence of ion intensity on SA%, others have reported that ion intensity is a major factor influencing overall quality of isotope ratio measurements.[13,19,25] For this reason, the SA% values that we obtained should be considered as a 'best case' scenario and for real-world applications, such as metabolite identification in complex biological matrices, SA% could be lower due to (1) insufficient ion intensity and/or (2) matrix peaks overlapping the isotopic profile of interest. Importantly, we also demonstrated that SA% determinations can alert the investigator when the analyte under investigation may exist as a mixture (1) due to the presence of structurally related components (e.g. dihydroquinidine) or (2) due to chemical or metabolic oxidation (e.g. troglitazone). In addition, this analysis may provide quantitative estimates as to the components of the mixture when the components are similar structurally. The results of our work demonstrate the utility of SA and should encourage manufacturers of mass spectrometers to determine the specifications of not only the mass accuracy, but also the spectral accuracy of their instruments.

Acknowledgements

We thank AB Sciex and Shimadzu for kindly lending the mass spectrometer and UFLC system used in this study. We thank Dr Yongdong Wang of Cerno Biosciences for creating Figs. 2 and 3 as well as helpful discussions on spectral accuracy.

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