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Comparison of negative ion electrospray mass spectra measured by seven tandem mass analyzers towards library formation

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A library of negative ion electrospray ionization mass spectra and tandem mass spectra (MS/MS) of sulfonated dyes has been developed for fast identification purposes. The uniform protocol has been elaborated and applied to the measurements of more than 50 anionic dyes. Three collision energies are selected in our protocol which ensures that at least one of them provides a suitable ratio of product ions to the precursor ion. The robustness is investigated with altered values of tuning parameters (e.g. the pressure of the nebulizing gas, the temperature and the flow rate of drying gas, and the mobile phase composition). The results of the inter-laboratory comparison of product ion mass spectra recorded on seven different tandem mass spectrometers (three ion traps, two triple quadrupoles and two hybrid quadrupole time of flight instruments) are presented for four representative anionic dyes – azo dye Acid Red 118, anthraquinone dye Acid Violet 43, triphenylmethane dye Acid Blue 1 and Al(III) metal-complex azo dye. The fragmentation patterns are almost identical for all tandem mass analyzers, only the ratios of product ions differ somewhat which confirms the possibility of spectra transfer among different mass analyzers with the goal of library formation.

Sulfonated dyestuffs are important industrial products with widespread applications produced annually in huge quantities. Due to the continuous development of new products with improved dyeing properties and a lower environmental impact, it is important to have suitable analytical tools for the identification and structural confirmation of new dyestuffs, their by-products, synthetic impurities and the degradation products found during the synthetic processes and in the environment. Ion-pairing high-performance liquid chromatography/mass spectrometry (HPLC/MS) with dihexyl- or triethylammonium acetate as ion-pairing agents has been used for the analysis of such compounds,[1,2] because it provides a good compromise between separation selectivity and mass spectrometric performance, but there are still some problems with signal suppression and memory effects.[3,4]

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Mass spectral libraries provide a powerful tool for the fast identification using both molecular weight (MW) and fragmentation information.[5] Gas chromatography/mass spectrometry (GC/MS) with electron ionization (EI) provides spectra rich in fragment ions that are specific for particular chemical structures. The main disadvantage of this approach is that many organic compounds are not amenable to GC without previous derivatization which is a serious limitation for non-volatile compounds. Another drawback of EI is excessive fragmentation for labile compounds which results in the total absence of a molecular ion. The introduction of atmospheric pressure ionization techniques has allowed the direct coupling of HPLC to MS, but the EI library approach cannot be adopted so easily mainly because of two reasons: (1) the lack of fragment ions in the full scan spectra and (2) unlike EI at 70 eV there are no universal conditions, the spectra depend strongly on the experimental conditions, such as the instrument type, ionization technique used (electrospray ionization (ESI), atmospheric pressure

chemical ionization (APCI) or atmospheric pressure photoionization (APPI)), the polarity mode (positive or negative) and also the mobile phase composition and the presence and concentration of additives.

The most suitable ionization technique for anionic dyes is ESI.[1–4] Other ionization techniques may also be applicable, always in the negative ion mode, for example APCI for dyes containing up to two sulfonic acid groups,[2,4] matrix-assisted laser desorption/ionization (MALDI),[6] or previously fast atom bombardment.[7–10] The strategy in the formation of libraries for soft ionization techniques is based on collision-induced dissociation (CID) either in the ion source region or preferably by tandem mass spectrometry (MS/MS) following the isolation of a selected precursor ion. The main complication in obtaining 'library-searchable' spectra is that a single set of conditions may not be applicable to a wide range of compounds. Moreover, the given set of conditions may produce different results on various instruments due to design differences between individual manufacturers. A multi-instrument study comparing APCI and ESI in-source CID on single quadrupole instruments from the same manufacturer reported large variations in the spectra of toxicologically relevant drugs.[11] A more extensive study involving six instruments from four manufacturers concluded that the source design of various instruments causes differences between the spectra of the same compound and neither of the two ionization techniques used in that study appeared to be universally transferable for all compounds within the test group across all instrument types.[12] The potential usefulness and applicability of in-source CID libraries were demonstrated on a group of about 800 pesticides and explosives[13] and in the general unknown screening of drugs and toxicants in serum.[14] Hough *et al.*[15] used the electrospray transport region of a HP 1100 MSD quadrupole mass spectrometer to generate library-searchable mass spectra at three offset voltages. A study of the effects of mobile phase composition, compound concentration and intra-laboratory reproducibility on in-source CID mass spectra led to the conclusion that reproducible spectra could be obtained over the defined range of instruments and conditions.[16–18]

The first reported library of tandem mass spectra was produced on a triple quadrupole mass spectrometer using single collision gas pressure and a single collision offset voltage and was applied to the identification of a limited range of pesticide residues in surface water.[19] Lopez *et al.*[20] reported reproducible spectra using wideband excitation and normalized collision energy with an ion trap mass spectrometer. The library on the ion trap, containing about 600 compounds of biomedical and environmental interest,[21] yielded highly reproducible and searchable spectra. A similar example of searchable MS/MS spectral libraries on the ion trap was reported for the analysis of flavonoids.[22] For multi-target drug screening, a MS/MS library based on product ion spectra using a hybrid triple quadrupole linear ion trap was successfully applied in real analyses.[23] The comparison of CID spectra obtained with different instruments is of major interest for the application of MS/MS libraries.[24] CID spectra of drugs were found to be highly reproducible between triple quadrupoles from the same manufacturer confirming the library transferability among the same instrument type in different laboratories. The comparison of product ion spectra acquired on the triple quadrupole mass spectrometers from two different manufacturers was published by Gergov *et al.*[25] The results indicated that after standardization of the instrumental conditions, HPLC/MS/MS spectral libraries of drug substances are suitable for inter-laboratory comparison. The effects of CID parameters on the library search were studied on ion trap and triple quadrupole instruments.[26] The presented results demonstrated that the mass spectral library searching algorithm, originally designed for databases of EI spectra, may be successfully applied for libraries of product ion spectra produced by low-energy CID on both triple quadrupole and ion trap instruments and the authors concluded that a single set of conditions could be suitable for obtaining library-searchable spectra on the condition that the collision energy is set above a threshold value. A more extensive comparison of MS/MS spectra recorded on three ion traps, one triple quadrupole and one Fourier transform ion cyclotron resonance mass spectrometer reported that MS/MS libraries could be independent of instrument type.[27]

The goal of the present work is the study of effects of individual tuning and collision parameters on MS/MS spectra and then the development of a robust procedure enabling the inter-laboratory transfer of libraries among different types of mass analyzers and diminishing known differences between the ion-trap-based and quadrupole-based analyzers. Data from seven different analyzers makes this the most comprehensive comparison so far reported in a single study.

EXPERIMENTAL

Materials

Acetonitrile (HPLC grade) was purchased from J.T. Baker (Deventer, The Netherlands). Water was deionised using a Demiwa 5-roi purification system (Watek, Ledeč nad Sázavou, Czech Republic) and an UltraClean UV apparatus (SG, Hamburg, Germany). All solvents were filtered through 0.45 μm Millipore filter prior to use. The samples of sulfonated dyes were obtained from the collection of Dr Josef Přikryl (Institute of Polymeric Materials, University of Pardubice, Czech Republic) and from Alliachem (Pardubice, Czech Republic).

Sample preparation

A set of more than 50 sulfonated dyestuffs with molecular weights in the range from 300 to 1200 Da containing one to five sulfonic acid and/or sulfate groups was selected for the initial construction of the library. These included, for example, dyes with the following Color Index (C. I.) names: Acid Black 1, Acid Blue 1, 22, 40, 43 and 81, Acid Brown 6, Acid Orange 5, 7, 8 and 10, Acid Red 1, 12, 14, 18, 26, 27, 88, 118 and 357, Acid Violet 7, 43 and 90, Acid Yellow 1, 23, 27, 36 and 194, Direct Black 17, Direct Blue 78, Direct Green 26 and 28, Direct Orange 7, Direct Red 7 and 20, Direct Yellow 28, Mordant Black 1, 3, 9, 11 and 15, Mordant Blue 23, Mordant Red 8, Mordant Yellow 1, 3 and 8, Pigment Red 1, Reactive Yellow 1 and 2, etc. All samples were dissolved in aqueous

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acetonitrile (50:50, v/v) to ensure both good solubility and ionization efficiency and introduced into each mass spectrometer either by direct infusion with a syringe pump or by the HPLC pump with an autosampler.

Instrumentation

An Esquire 3000 ion trap (Bruker Daltonics, Bremen, Germany) instrument was used for the initial method development. The sample solution was introduced into the ion source using the syringe pump (COLE Palmer Instrument Company, Vernon Hills, IL, USA) under the following conditions: full scan and tandem negative ion ESI mass spectra were recorded over the mass range *m/z* 50–800, the flow rate was 5 μL/min, the capillary voltage 3 kV, the nebulizing gas pressure 10 psi, the drying gas flow rate 4 L/min, the drying gas temperature 300°C, and the tuning parameter target mass *m/z* 500. A precursor ion isolation width of 4 *m/z* units was used for all the MS/MS experiments. The seven tandem mass analyzers used in the inter-laboratory comparison (details of experimental conditions are given in Table 1) were: (1) Esquire 3000 ion trap (Bruker Daltonics), (2) Esquire LC ion trap (Bruker Daltonics), (3) LCQ ion trap (ThermoElectron, San Jose, CA, USA), (4) 1200L triple quadrupole (Varian, Palo Alto, CA, USA), (5) Quattro Premier triple quadrupole (Waters Micromass, Manchester, UK), (6) QqTOF Ultima API (Waters Micromass), and (7) QqTOF MicrOTOF-Q (Bruker Daltonics).

Operating conditions

Based on our previous experiences on the collisional stabilities of sulfonated dyes,[1,2,28] three collision energies (0.6 V = 2/3X, 0.9 V = X and 1.2 V = 4/3X) were selected for the experiments and data acquisition using the Esquire 3000 ion trap. The middle collision energy (0.9 V = X) was found to be an optimal value for yielding a reasonable ratio between the abundances of the $[M–H]^-$ precursor ion and the product ions. The higher collision energy (1.2 V = 4/3X) was used to ensure the fragmentation of very stable compounds and to obtain low-mass product ions, while the lower collision energy (0.6 V = 2/3X) was suitable for labile compounds (dyes containing one or more sulfate groups, e.g. the group of Reactive dyes). Other tuning parameters (e.g., pressure of nebulizing gas, temperature and the flow rate of the drying gas) were kept constant in all experiments, as summarized in Table 1.

Protocol for the method transfer among tandem mass analysers

The procedure for the method transfer among participating laboratories with different tandem mass analyzers is summarized here. First, the background signal is checked with the same solvent mixture as for the measurement. The collision energy (X) is set to obtain the ratio of 100/12 ± 2% for the relative abundances of the product ion at *m/z* 355 and of the precursor ion at *m/z* 539 for the model compound C.I. Acid Red 118. Then, relative abundances are recorded ten consecutive times during at least 1 min data averaging, and the arithmetic mean and the mean deviation are calculated. The same procedure is repeated for the other two collision energies, equal to 2/3X and 4/3X. When the initial protocol is transferred to another instrument, the optimal collision energy X has to be found on the basis of the same ratio of the precursor ion $[M–H]^-$ and the most abundant product ion *m/z* 355 of the model compound C.I. Acid Red 118. The higher (4/3X) and lower (2/3X) collision energies are then calculated and data acquisition is repeated under the new conditions. The identical conditions are used for the measurement of all compounds in the library.

Table 1. List of tuning parameters including collision energies for all tandem mass spectrometers used in this study

	Collision energy			
Instrument	2/3X	X	4/3X	Tuning parameters[a]
(1) Esquire 3000 ion trap	0.6 V	0.9 V	1.2 V	Capillary voltage 3 kV, nebulizing gas pressure 10 psi, drying gas flow rate 4 L/min, drying gas temperature 300°C, target mass *m/z* 500
(2) Esquire LC ion trap	0.56 V	0.84 V	1.12 V	Capillary voltage 3 kV, nebulizing gas pressure 10 psi, drying gas flow rate 4 L/min, drying gas temperature 300°C, target mass *m/z* 500
(3) LCQ ion trap	0.69 V	1.035 V	1.38 V	Capillary voltage 4.5 kV, nebulizing gas flow rate 40 arbitrary units, entrance capillary temperature 220°C, entrance capillary voltage −47.65 V
(4) 1200L triple quadrupole	18.33 eV	27.5 eV	36.67 eV	Capillary voltage 3.5 V, nebulizing gas pressure 25 psi, drying gas flow rate 4 L/min, drying gas temperature 300°C
(5) Quattro Premier triple quadrupole	20 V	30 V	40 V	Capillary voltage 3.2 kV, source temperature 80°C, desolvation temperature 200°C, desolvation gas flow rate 149 L/h, cone gas flow rate 14 L/h
(6) QqTOF Ultima API	22 V	33 V	44 V	Source temperature 80°C, desolvation temperature 200°C, cone gas flow 50 L/h, desolvation gas flow rate 500 L/h
(7) QqTOF micrOTOF-Q	18.66 eV	28 eV	37.33 eV	Capillary voltage 4.5 kV, nebulizing gas pressure 0.5 bar, drying gas flow rate 4 L/min, drying gas temperature 180°C

[a] Individual terms are used in accordance with particular manufacturers, e.g. the collision energy is mostly expressed in V, while other manufacturers use values in eV.

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RESULTS AND DISCUSSION

Method development with the Esquire 3000 ion trap

The anionic dyes (i.e., sulfonated, sulfated and metal-complex dyes) chosen for this study represent dyes widely used in the textile industry. The full scan negative ion mass spectra of sulfonated dyes show deprotonated molecules $[M–H]^-$ in the case of monosulfonated dyes or series of deprotonated molecules $[M–xH]^{x-}$ and their adducts with sodium ions $[M–(x+y)H+yNa]^{x-}$ in the polysulfonated dyes; these ions can be used for the MW determination.[4,28] When a sulfate group is present (e.g. the group of Reactive dyes), the MW determination becomes more difficult due to the extensive fragmentation already occurring in the full scan mode. Moreover, the sulfate group is usually present in the form of a sodium salt (similarly to the sulfonic acid group), so the mass spectra show abundant adducts with sodium ions. Four dyes as representatives of important industrial groups of dyes were selected for inter-laboratory comparison (Fig. 1), azo dye C. I. Acid Red 118, anthraquinone dye C. I. Acid Violet 43, triphenylmethane dye C. I. Acid Blue 1 and Al(III) metal-complex azo dye. First, the optimization of tuning parameters was performed (see Experimental section) and then full scan and tandem mass spectra were measured under optimized conditions and imported into two libraries. The first library contained the full scan spectra, while the second one was used for product ion spectra measured at three collision energies. The full scan mass spectra cannot provide enough information for identification and they may be also influenced by the different type and relative abundances of adducts due to varying content of salts in the solution and differences among the geometries of ion sources from different vendors. The full scan mass spectra library provides supplementary information for the confirmation of MW determination, especially for polysulfonated/polysulfated dyes, because their full scan mass spectra are rather complex,[1,2,28,29] containing series of multiply charged ions $[M–xH]^{x-}$ and $[M–(x+y)H+yNa]^{x-}$. The second library also contains supplementary information, such as MWs, Color Index (C. I.) names, molecular formulae and structures.

One monosulfonated dye with a medium MW was chosen as a model compound for the optimization of standard conditions for acquiring product ion spectra into the library and to study the influence of tuning and collision parameters on MS/MS spectra. The structures of model compound C.I. Acid Red 118 and other three model compounds are depicted in Fig. 1. The standard set of conditions should provide a reasonable ratio between the precursor $[M–H]^-$ ion and abundant product ions. Such a collision energy is designated as 'X'. The full scan mass spectrum of C. I. Acid Red 118 shows only the $[M–H]^-$ ion at *m/z* 539 (spectrum not shown). MS/MS spectra at three different collision energies are shown in Fig. 2. When the low collision energy (0.6 V = 2/3X, Fig. 2(a)) is used, only three product ions (*m/z* 459, 355 and 250) are observed together with the deprotonated molecule. Higher values of collision energy (Figs. 2(b) and 2(c)) increase the number of product ions, but their absolute signal intensities decrease. The so-called 'cut-off phenomenon', where ion traps cannot efficiently retain product ions with *m/z* values lower than about 30% of *m/z* value of the precursor ion, may influence the relative abundances of

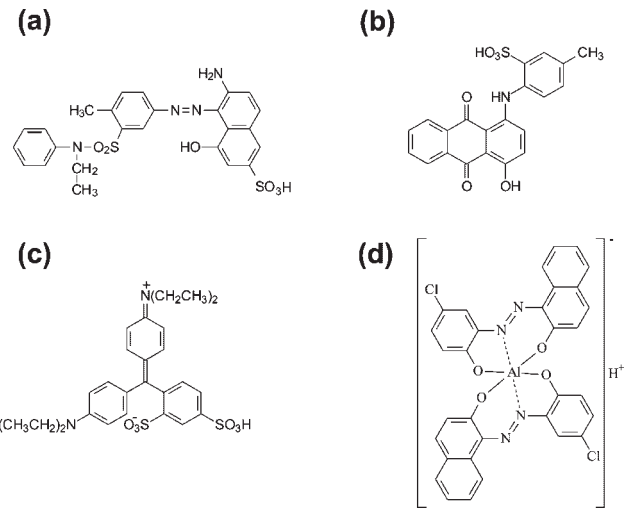

Figure 1. Structures of four model compounds: (a) azo dye C. I. Acid Red 118 (MW 540), (b) anthraquinone dye C. I. Acid Violet 43 (MW 409), (c) triphenylmethane dye C. I. Acid Blue 1 (MW 544), and (d) Al(III) metal-complex azo dye (MW 620).

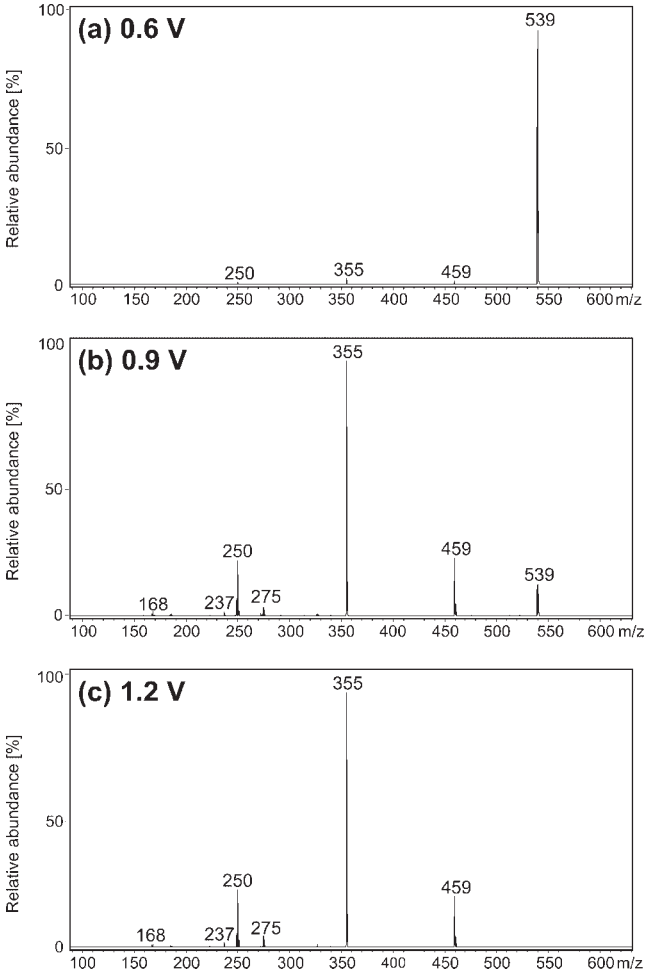

Figure 2. Negative ion ESI-MS/MS spectra of the precursor ion $[M–H]^-$ at *m/z* 539 using three collision energies measured on the Esquire 3000 ion trap mass spectrometer: (a) 0.6 V (=2/3X), (b) 0.9 V (=X), (c) 1.2 V (=4/3X).

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Table 2. Reproducibility of MS/MS spectra of the model compound C.I. Acid Red 118 acquired with the Esquire 3000 ion trap mass analyzer in ten consecutive measurements averaged over 1 min

	Relative abundances [%] for three collision energies		
m/z	2/3X (=0.6 V)	X (=0.9 V)	4/3X (=1.2 V)
539	100 ± 0	12.54 ± 1.1	0 ± 0
459	1.36 ± 0.2	22.12 ± 1.7	19.73 ± 0.9
355	3.62 ± 0.7	100 ± 0	100 ± 0
275	0 ± 0	4.97 ± 1.0	4.75 ± 0.4
250	0.91 ± 0.2	22.03 ± 0.9	22.26 ± 0.7
237	0 ± 0	1.86 ± 0.4	1.97 ± 0.4
168	0 ± 0	0.96 ± 0.3	0.78 ± 0.2

product ions with such *m/z* (*m/z* 168 in this example). It also has a relatively strong effect on the product ions slightly above that limit (e.g. *m/z* 237 and 250). This phenomenon results in reduced relative abundances of low-mass product ions in comparison with what is obtained when using QqQ and QqTOF tandem mass analyzers. In addition to the cut-off phenomenon, the different activation mechanism in ion-trap-based devices from that in quadrupole-based collision cells also plays a significant role.

Table 2 illustrates very good reproducibility of relative abundances of product ions. The relative standard deviation calculated from ten consecutive injections is always better than or equal to 1.7%. Similar results on robustness are obtained for other model dyes. The robustness of the method was investigated with altered values of parameters which may influence MS/MS spectra. The effects of eight parameters were studied in the range of standard values (±50%) recommended by the manufacturer: nebulizing gas pressure, drying gas flow rate, drying gas temperature, capillary voltage, skimmer voltage, trap drive voltage, solvent composition and flow rate. No measurable and statistically relevant effects of those parameters on the MS/MS spectra of model compounds at any of three collision energies were found but, of course, strong effects on the sensitivity were observed, when the tuning parameters were far from the optimal values. The ratios of the relative abundances of product ions to the precursor ion were not affected by changed tuning parameters, as initially expected.

Comparison of tandem mass spectra measured on different analysers

The degree of fragmentation variability of model compounds on seven tandem mass analyzers is illustrated in Fig. 3. Comparison of MS/MS spectra measured on the seven different mass spectrometers indicates that the product ions mostly have the same *m/z* values. In some cases, the masses observed on ion traps are shifted by 1 *m/z* unit compared with those on QqQs and QqTOFs, e.g. *m/z* 168 vs. 167, *m/z* 237 vs. 236, which corresponds to the formation of odd-electron ions instead of even-electron ions. This shift can be probably explained by the different methods of precursor ion activation. Frequency activation in the ion trap is rather selective for the precursor ion, while collision activation in the quadrupole collision cell can cause the fragmentation of

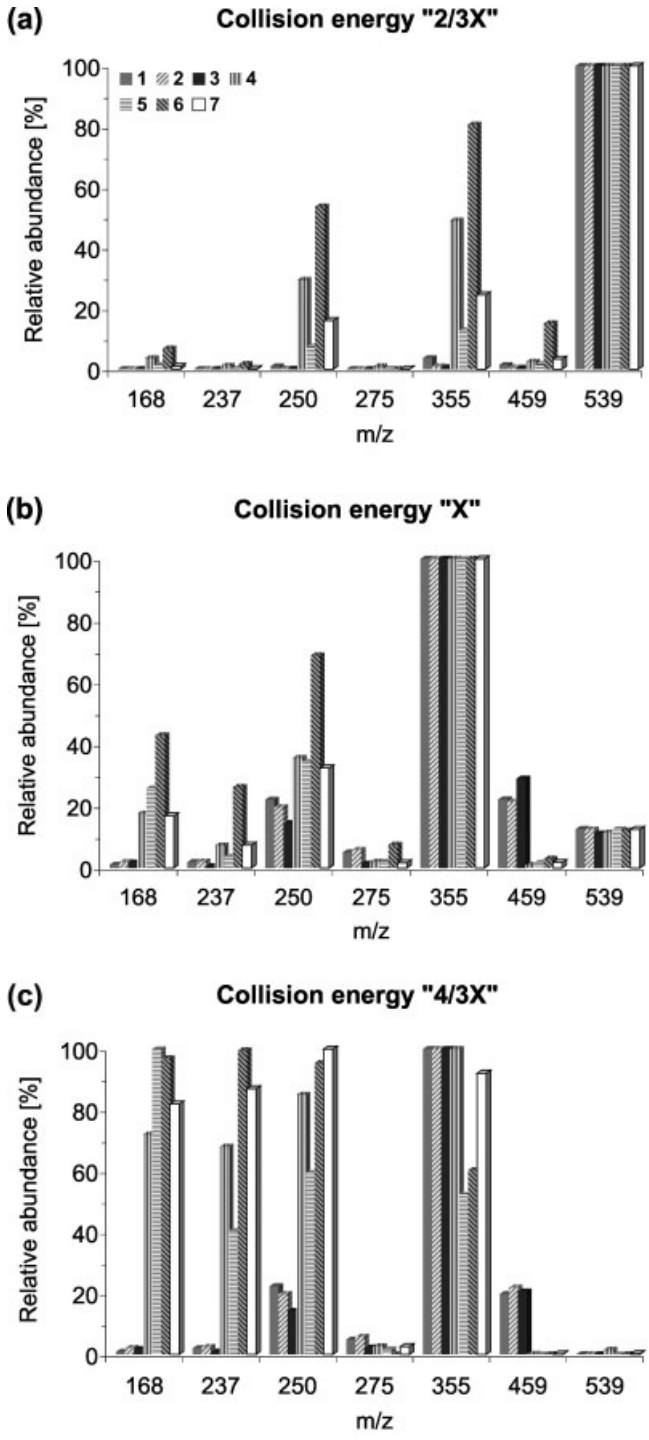

Figure 3. Results of inter-laboratory comparison of MS/MS spectra of the $[M–H]^-$ ion of model compound C.I. Acid Red 118 measured with three collision energies and seven different tandem mass analyzers: (a) collision energy X, (b) collision energy 2/3X, (c) collision energy 4/3X; (1) Esquire 3000 ion trap, (2) Esquire LC ion trap, (3) LCQ ion trap, (4) 1200L triple quadrupole, (5) Micromass Quattro Premier triple quadrupole, (6) QqTOF Ultima API, and (7) QqTOF MicrOTOF-Q.

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the precursor and the product ions, resulting in a different overall appearance of the MS/MS spectra. MS/MS spectra acquired with three ion traps from two different manufacturers exhibit highly comparable spectra for all studied dyes with only small differences in the relative abundances of individual ions (see Figs. 3–5). QqQ tandem mass spectra are typically more rich in product ions over the whole mass range. The relative abundances can vary significantly when comparing MS/MS spectra of model compounds using QqQ and QqTOF mass spectrometers with those obtained using ion traps; in some multifunctional dyes additional product ions were observed in the low-mass region in QqTOF measurements, e.g. the $[SO_3]^{-\cdot}$ ion at *m/z* 80. To improve the inter-laboratory comparison of MS/MS spectra, the arithmetic (Fig. 4(a)) and weighted (Fig. 4(b)) averages of relative abundances of product ions were calculated. For the weighted average, 50% of the relative abundance at collision energy X and 25% of the relative abundances at collision energies 2/3X and 4/3X were taken for the calculation. The resulting values were normalized to 100%. This procedure provides comparable and transferable results for most studied dyes but, in fairness, it should be noted that about 20% of complex dyes with higher MWs and multiple functional groups may provide a significantly higher degree of fragmentation on QqQ and QqTOF instruments. This is not surprising when we consider the huge structural diversity of industrial dyestuffs.

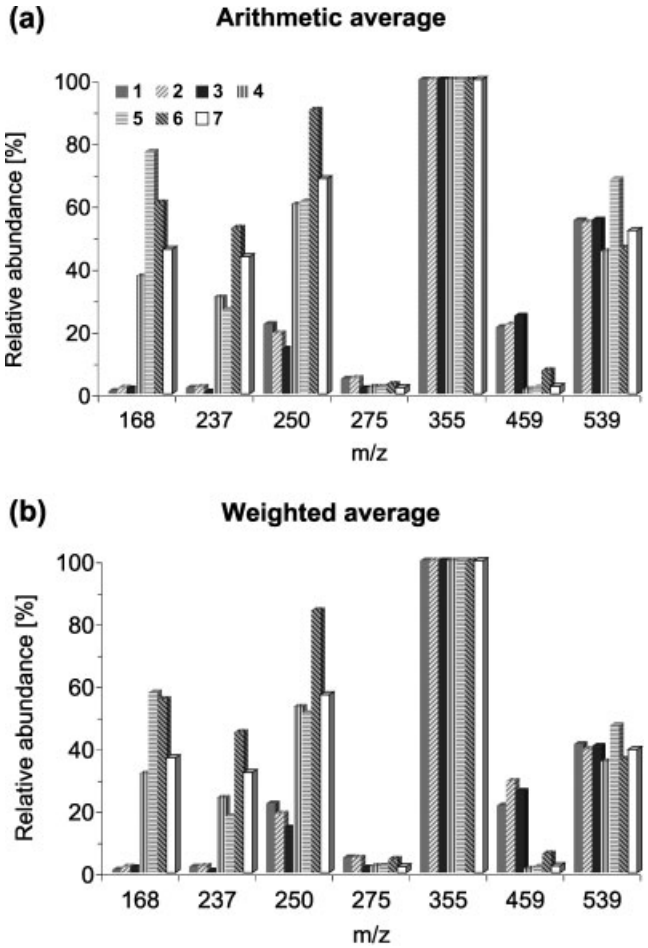

Figure 4. Results of inter-laboratory comparison of MS/MS spectra of the $[M–H]^-$ ion of model compound C.I. Acid Red 118 measured on seven different tandem mass analyzers: (a) arithmetic average of signal intensities at three collision energies, (b) weighted average (X = 50%, 4/3X = 25% and 2/3X = 25%) of intensities at three collision energies. (1) Esquire 3000 ion trap, (2) Esquire LC ion trap, (3) LCQ ion trap, (4) 1200L triple quadrupole, (5) Micromass Quattro Premier triple quadrupole, (6) QqTOF Ultima API, and (7) QqTOF MicrOTOF-Q.

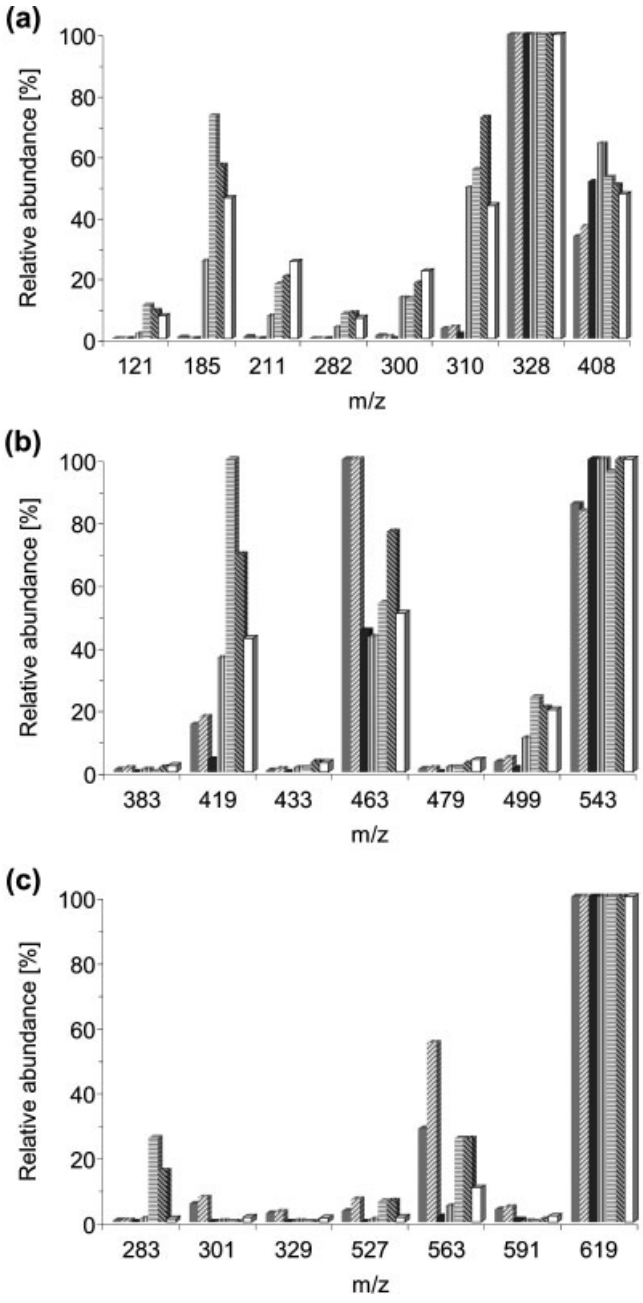

Figure 5. Weighted averages (X = 50%, 4/3X = 25% and 2/3X = 25%) of intensities at three collision energies for three representatives of dye classes: (a) C. I. Acid Violet 43 as anthraquinone dye, (b) C. I. Acid Blue 1 as triphenylmethane dye, (c) Al(III) metal-complex azo dye. (1) Esquire 3000 ion trap, (2) Esquire LC ion trap, (3) LCQ ion trap, (4) 1200L triple quadrupole, (5) Micromass Quattro Premier triple quadrupole, (6) QqTOF Ultima API, and (7) QqTOF MicrOTOF-Q.

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The role of the three different collision energies is demonstrated on examples of very stable compounds which do not fragment easily (e.g. non-sulfonated metal-complex dyes)[30] or very labile compounds (e.g. Reactive dyes containing one or more sulfate groups).[28] For an extremely stable molecule (e.g. the metal-complex dye in Fig. 5), only the highest collision energy 4/3X provides some product ions although with low relative intensity, but almost no fragmentation is apparent for lower collision energies, 2/3X and X. On the other hand, only the lowest collision energy 2/3X provides meaningful spectra for extremely labile compounds (e.g. polysulfated dyes) which tend to fragment extensively even in the full scan mode (e.g. disulfated dyes in Fig. 2, Ref.[28]). In such rare cases, the selection of an appropriate precursor ion and subsequent MS/MS measurement is complicated. In this case the library of full scan spectra is a valuable source of information in addition to the MS/MS library.

The presented results show that different mass analyzers provide transferable MS/MS spectra useful for library formation, but that some differences in relative abundances of individual product ions should be expected in a highly predictable manner. First, ion traps have significantly decreased relative abundances of product ions below the cut-off (i.e. 1/3 of precursor ion *m/z* value), but ions close to the cut-off value are also slightly affected. In addition, some very low-mass product ions can be completely missing, e.g. $[SO_3]^{-\bullet}$ at *m/z* 80. Secondly, QqQ and QqTOF instruments provide somewhat decreased relative abundances of product ions in the high-mass region close to the precursor ion compared with ion traps. This is primarily caused by the fact that the quadrupole collision cell can provide collision gas activation for the precursor ion and the formed product ions resulting in multiple fragmentation events for the precursor ion. On the contrary, ion-trap-based analyzers rely on the use of selective frequency activation only for a certain relatively narrow interval of *m/z* masses around the precursor ion; therefore, product ions should not be affected and single-step fragmentation resulting in the lack of product ions in the low-mass region is mostly observed. Another reason for the reduced abundances of product ions in the low-mass region is the poorer stabilization of low-mass ions in the ion trap, described by the cut-off effect. Regardless of certain differences in relative abundances, our comparison shows that the identification approach for a defined range of compounds (in this case anionic organic dyes) using our in-house mass spectrometric library with a soft ionization technique, negative ion ESI, in the MS/MS mode is meaningful.

CONCLUSIONS

The comparison of tandem mass spectra measured on seven different types of mass analyzers confirms known differences between the relative ratios of product ions for individual instruments, mainly between ion-trap- and quadrupole-based analyzers. In principle, it is impossible to unify the relative abundances of product ions measured by different ways of activation, but somewhat different relative abundances are not a serious obstacle to the transferability of libraries. Our approach is based on measurements at three different collision energies, the first value of collision energy 'X' is optimal for most compounds, an increased collision energy '4/3X' is suitable for very stable compounds and for obtaining low-mass product ions even with ion traps, to partially overcome the absence of low-mass product ions caused by the different activation mechanism and the cut-off effect, and finally the decreased collision energy '2/3X' is selected for very labile compounds and it also increases relative abundances with QqQ and QqTOF instruments in the high-mass region close to the *m/z* value of the precursor ion. A better correlation among different analyzers can be obtained by averaging the relative abundances at the three collision energies or even better by weighted averaging. The applicability of our approach is demonstrated on four model compounds as representatives of different classes of commercial dyestuffs.

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