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Evaluation of the performance of a tandem mass spectral library with mass spectral data extracted from literature

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MSforID represents a database of tandem mass spectral data obtained from (quasi-)molecular ions produced by atmospheric pressure ionization methods. At the current stage of development the library contains 12 122 spectra of 1208 small (bio-) organic molecules.

The present work was aimed to evaluate the performance of the MSforID library in terms of accuracy and transferability with a collection of fragment ion mass spectra from various compounds acquired on multiple instruments. A literature survey was conducted to collect the set of sample spectra. A total number of 554 spectra covering 291 compounds were extracted from 109 publications. The majority of spectra originated from publications on applications of LC/MS/MS in drug monitoring, pharmacokinetics, environmental analysis, forensic analysis as well as food analysis. Almost all types of tandem mass spectrometric instruments distributed by the five most important instrument vendors were included in the study.

The overall sensitivity of library search was found to be 96.4%, which clearly proves that the MSforID library can successfully handle data from a huge variety of mass spectrometric instruments to allow accurate compound identification. Only for spectra containing three or more fragment ions, however, the rate of classified matches (= matches with a *relative average match probability (ramp)* score $>$ 40.0) was 95%. Ambiguous or unclassified results were mainly obtained for searches with single precursor-to-fragment ion transitions due to the insufficient specificity of such a low amount of structural information to unequivocally define a single compound. Copyright © 2011 John Wiley & Sons, Ltd.

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Keywords: tandem mass spectrometry; mass spectral library; identification; MsforID

Introduction

Tandem mass spectrometry (MS/MS) implementing collision induced decomposition (CID) is a well established method for the generation of fragment ions.[1] Typically, (quasi-)molecular ions obtained from atmospheric pressure ionization techniques are selected and fragmented in inert gas-filled collision cells. The mass-to-charge ratios (*m/z*) as well as the intensities of the resulting ions are recorded to obtain tandem mass spectra. These spectra usually represent characteristic fingerprints for molecular structures that can be used for compound identification.

For automated identification of small (bio-)organic molecules compound-specific fragmentation patterns can be stored in tandem mass spectral libraries. Applications of these libraries include the identification of pharmaceutical compounds, illegal drugs, and pesticides.[2–12] For a long period of time the development and the widespread use of such libraries was hampered by the limited reproducibility of fragmentation. Fragmentation patterns turned out to be strongly influenced by experimental conditions such as collision energy, collision gas (pressure and type), as well as instrument design.[13–15] Accordingly, identification with tandem mass spectral libraries worked well only in the laboratory and on the instrument where the library had been established, and the statistical sensitivity for library search with spectra obtained from different sources was usually lower than 80%.[16]

Several strategies were developed to improve accuracy, robustness and transferability. One strategy was based on the use of tuning point protocols to standardize CID conditions between instruments.[17–19] Reference compounds were used to tune instruments so that the spectrum of the tune compound matches its own spectrum acquired on the reference instrument. Sensitivities reported for this approach were found to be $<$85%.[20] To some extent tuning protocols improved performance, but did not convert tandem mass spectrometric libraries into universal identification tools. The approach most widely adopted for the acquisition of reference spectra is the use of several collision energies that cover a broad range of the specific breakdown curve of a reference compound.[6,8,21] Particularly in combination with tailor-made search algorithms this approach turned out to yield good performance even with datasets acquired on various instruments.[6,22–25] In this context the MSforID project (www.msforid.com) represents one of the most promising approaches towards an accurate, robust and transferable tandem mass spectral library.[6,22,23,25]

The MsforID library was established on a high-resolution quadrupole – quadrupole – time-of-flight (QqTOF) instrument using ten different collision energies for acquiring reference spectra specific for a certain compound. At the current stage of development the library contains 12 122 spectra of 1208

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compounds. The tailor-made search algorithm is based on peak matching and is capable to handle tandem mass spectral libraries that contain multiple spectra per compound. The search algorithm exhibits a high tolerance towards changes within the intensity distribution among different fragmentation pathways. During library search, the similarity between a measured spectrum and each set of compound-specific reference spectra is determined, and a numerical value (relative average match probability, *ramp*) is provided as output that can be used to classify a match as correct positive or false positive. The performance of MSforID has been tested in two studies.[22,23,25] In a multicenter study 22 test compounds were sent to three different laboratories, where 418 tandem mass spectra were acquired using four different instruments from two manufacturers. With this data set sensitivity was found to exceed 95%. In a second study the Weinmann library containing over 5600 spectra of 1253 compounds acquired on a quadrupole–quadrupole–linear ion trap instrument (QqLIT) was used as sample set that was matched to the MSforID library. The obtained sensitivity exceeded 95% as well.

Extending our recent publications we herein report results obtained from matching tandem mass spectra extracted from literature to the MSforID library. The study was aimed to test the reliability of match as well as the transferability of the MSforID library. Due to the common use of liquid chromatography (LC)-MS/MS for the qualitative and quantitative analysis of (bio-)organic molecules, literature was considered to contain tandem mass spectral information for a large variety of compounds acquired on diverse instruments. Overall 554 spectra of 291 compounds were collected and matched. Almost all common mass spectrometric instruments distributed by the five most important instrument manufacturers were covered by the study. A large portion of the spectra contained only one, two, or three fragment ions per spectrum allowing assessment of the impact of the number of informative features on the performance of library search. Finally, the applicability of the MSforID library to check the selectivity of precursor-to-fragment ion transitions was examined.

Experimental

Reference library

The MSforID library was developed on a QqTOF instrument (Qstar XL, AB Sciex, Foster City, CA, USA) using electrospray ionization in positive and negative ion mode. A detailed description of the instrumental parameters applied can be found elsewhere.[6,9] For each reference compound, product-ion spectra were typically acquired at ten different collision energy values between 5 eV and 50 eV. Because of possible saturation effects and to avoid false positive matching of the precursor ion with product ions originating from alternative compounds, all signals within a ±4.0 amu window around the *m/z* of the precursor ion were deleted from the reference spectra obtained. Low abundant and unspecific signals were removed from reference spectra by filtering.[6,23] At the current stage of development the library contains 12 122 spectra of 1208 compounds. A more detailed description of the mass spectral library is provided on www.msforid.com. The library will be published as *Wiley Registry of Tandem Mass Spectral Data, MSforID*.[26] For this study the collection of spectra acquired in the positive ionization mode (10 712 MS/MS-spectra of 1040 compounds) was used as reference library.

Literature data

Literature was surveyed via PubMed search for tandem mass spectral data of compounds covered by the MSforID library. The search was focused on spectra obtained from CID of $[M+H]^+$-ions produced either by electrospray or atmospheric-pressure chemical ionization. A total of 590 tandem mass spectra were collected. Thirty-six spectra (6.1%) were discarded. Out of these 36 spectra, 28 spectra were found to be improperly calibrated (m/z-error $>$ 0.25). In the remaining eight spectra excluded major inconsistencies (i.e. wrong precursor ion, no spectral overlap) between the provided data and the corresponding reference spectra were observed. Five hundred and fifty-four spectra covering 291 compounds were included in the study. A detailed list of the compounds covered can be found in Supporting Information Table 1. The 554 spectra were extracted from 109 publications, which were published between 1998 and 2010 (Figure 1a). The publications are listed in Supporting Information Table 2. The publications covered different fields of applications such as drug monitoring/pharmacokinetics, environmental analysis, forensic analysis, food analysis and others (Figure 1b). Six types of mass spectrometers from five different manufacturers (AB Sciex, Foster City, CA, USA; Thermo Scientific, Waltham MA, USA; Waters, Milford, MA, USA; Agilent, Santa Clara, CA, USA; Bruker Daltonics, Bremen, Germany) were used to generate the fragment ion mass spectra (Figure 2). The following types of instruments were covered: quadrupole–quadrupole–quadrupole (QqQ), QqLIT, ion trap (IT), QqTOF, linear ion trap–Orbitrap (LIT-Orbitrap), and quadrupole (Q) with in-source CID. The extracted spectra were categorized based on the number of fragment ions provided. The following categories were used: spectra with one fragment, spectra with two fragments, spectra with three fragments, and spectra with more than three fragments.

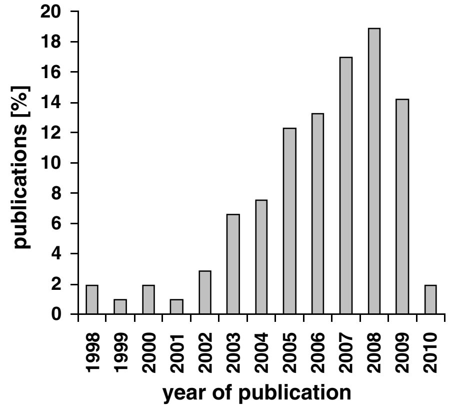
Data handling

Extracted mass spectra were used as input for search within the MSforID library. Mass spectrometric information needed for library search included the *m/z* of the precursor ion as well as the list of observed fragment ions (*m/z* and the corresponding relative intensities). To estimate the frequency of single precursor-to-fragment ion transitions shared by different compounds, all transitions extracted from literature were matched individually to the library. For this experiment only spectra containing one, two or three fragment ions were considered (775 transitions out of 453 spectra).

Table 1. Statistical evaluation of the reliability of match for spectra extracted from literature.

Category	Number of spectra matched	Correct assignments [%] with	
		ramp > 0	*ramp* > 40
1 fragment/spectrum	205	93.2	80.5
2 fragments/spectrum	174	97.7	86.8
3 fragments/spectrum	74	100	94.6
>3 fragments/spectrum	101	98.0	96.0

(a) year of publication



(b) application areas

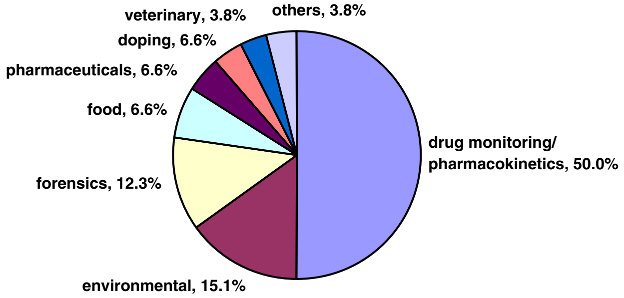
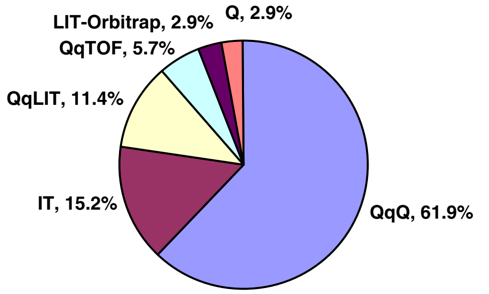


Figure 1. Percentage distributions of publications covered by this study (a) over the years of publication and (b) over application areas.

The principles of the applied library search algorithm have been described elsewhere.[6,22,23] Briefly, the measured product ion mass spectrum of an unknown compound represents the input for library search. The spectrum is compared with all mass spectra stored in the library. In each case the similarity is determined. The estimation of similarity starts with the identification of fragment ions that are present in both of the two spectra compared. They are called 'matching fragments'. The spectral information retrieved is used to calculate the 'reference spectrum-specific match probability' (*mp*). As the mass spectral library consists of MS/MS-spectra that have been collected at several different collision energy values, for each single reference compound several *mp*-values are obtained. To combine all reference compound-specific *mp*-values to one value that specifies the similarity between the unknown and the specific reference compound, the reference compound-specific *mp*-values are averaged and normalized to yield the compound-specific 'relative average match probability' (*ramp*). Single *ramp*-values range between 0 and 100. A high compound-specific *ramp*-value indicates high similarity between the unknown and the reference compound. The substance with the highest *ramp* is considered to represent the unknown compound. The *ramp*-value is used to classify a match as correct positive or false positive. Recently, we could show that a good compromise between sensitivity and specificity can be obtained using a *ramp*-threshold of 40–50.[23,25] As final qualifying criterion to exclude putative false positive hits, the *m/z* of the precursor ion is used. Only if the *m/z* of the best matching compound agrees with the *m/z* of the precursor ion, identity is confirmed.

(a) instrument types



(b) instrument vendors

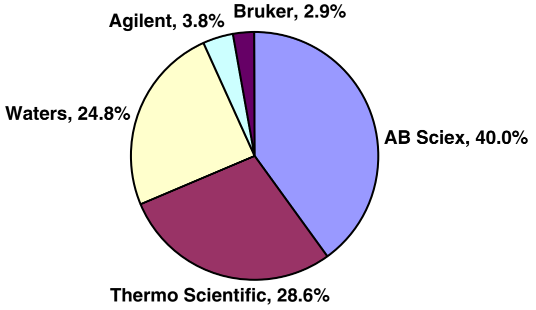


Figure 2. Percentage distributions of mass spectrometric instruments covered by this study (a) over the types of mass analyzers and (b) over instrument vendors.

Automated library search was performed with a program written in ActivePerl 5.6.1 (Active State Corporation, Vancouver, Canada) using the following search parameters: mass tolerance of ±0.25, cut-off factor of 0.05. Calculations were performed on a personal computer running Windows XP operating system (1.7 GHz Pentium, 1.0 GB RAM). Run time for a single spectral match was $<$0.2 seconds.

Results and discussion

Characterization of the data set

We have conducted a literature survey and searched for published tandem mass spectrometric data on compounds covered by the MSforID library. Literature was considered to represent a completely independent resource of tandem mass spectrometric data ideally suited to test the sensitivity of the MSforID library. Focus of the search was on CID of $[M+H]^+$-ions produced by atmospheric pressure ionization techniques. A total of 590 tandem mass spectra were collected. Thirty-six spectra (6.1%) were discarded. In 28 spectra, unacceptably large deviations between published *m/z*-values and the corresponding reference values (*m/z*-error $>$ 0.25) were observed. In the remaining eight spectra excluded major inconsistencies between the published data and the corresponding reference spectra were found. Wrong precursor ion masses and missing spectral overlap represented disqualifiers. The unexpectedly high percentage of faulty spectra (6.1%) clearly indicates a need for a more extended and a more carefully performed quality control of tandem mass spectral data during method development, manuscript preparation, and peer-reviewing. In this context tandem mass spectral libraries such as the MSforID library could represent valuable tools for authors, reviewers and editors to check the correctness of the provided mass spectral information prior to publication.

Five hundred and fifty-four spectra of 291 compounds were included in the study. Almost 28% of all compounds included in the MSforID library were covered by the study. The spectra were extracted from 109 publications (Supporting Information Table 2). The publications covered different fields of applications that make use of MS/MS either for qualitative or quantitative analysis. A summary of the application areas is provided in Figure 1b. As at the current stage of development the majority of entries in the MSforID library are different kinds of pharmaceutical compounds, publications on drug monitoring and pharmacokinetics represented the main sources of sample spectra (50.0%). Other important application areas were environmental analysis (15.1%), forensic analysis (12.3%), as well as food (6.6%) and doping analysis (6.6%).

The mass spectra included in this study were acquired on six different types of instruments (Figure 2a). Almost all major configurations that can be used to generate and scan fragment ions were included. The frequency of usage of the different instrument types in analytical laboratories is reflected by the percentage distribution provided in Figure 2a. The majority of instruments applied have QqQ configuration (61.9%). Mainly due to their low costs of purchase and due to their satisfying detection sensitivity, QqQ instruments are commonly used particularly for LC/MS/MS applications. Ion trap instruments represent another important class of mass spectrometers. Both IT (15.2%) and QqLIT (11.4%) are of interest due to their MS^n capabilities. Medium- to high-resolution instruments (QqTOF, 5.7%; LIT-Orbitrap, 2.9%) are gaining more and more interest in the fields of applications described above. Currently, however, the rather high purchase costs seem to represent a major obstacle for a more widespread use of such instruments.

The mass spectrometric instruments used to generate fragment ion mass spectra collected were purchased from five different manufacturers (AB Sciex, Thermo Scientific, Waters, Agilent, Bruker Daltonics, Figure 2b). Market leaders in the small molecule segment seem to be AB Sciex, Thermo Scientific, and Waters. AB Sciex instruments included different types of QqQ and QqLIT instruments. The most commonly applied mass spectrometers from Thermo Scientific had QqQ- or IT-configurations. Waters mainly contributed QqQ instruments.

The majority of spectra collected for this study originated from publications on LC-MS/MS. As authors often only report the fragment ions used to develop their method and do not provide full MS/MS information, the number of fragments available per spectrum was in many cases truncated to one, two, or three ions. As we anticipated that the number of fragment ions could have an impact on the sensitivity of library search, extracted spectra were categorized based on the number of fragment ions provided. The following categories were established: spectra with one fragment, spectra with two fragments, spectra with three fragments, and spectra with more than three fragments.

Evaluation of the search performance

Five hundred and fifty-four spectra of 291 compounds were matched to the MSforID library. In each case the five top matching compounds and the corresponding *ramp*-values were retrieved as output. In 534 cases, the correct compound was ranked first. The overall sensitivity was 96.4%, which clearly proves that the MSforID library can successfully handle data from a huge variety of mass spectrometric instruments to allow accurate compound identification. In only 20 cases (3.6%), the correct compound was ranked second. Six out of these 20 spectra (1.1%) contained more than one fragment ion per spectrum. These spectra were obtained from morphine, codeine, and doxycycline, and retrieved hydromorphone, hydrocodone, and tetracycline as top matching compounds. In each case the pairs of top matching compounds showed a high degree of structural similarity, which gave rise to overlapping fragmentation patterns and ambiguous search results. The remaining 14 spectra affected (2.5%) belonged to the group of spectra that contained only one fragment ion per spectrum. Among those a significant amount of spectra were mismatched due to the fact that two or more structurally diverse compounds may occasionally share the same precursor-to-fragment ion transition.

The fact that spectra with a low number of fragment ions were more likely giving rise to ambiguous results suggests that search performance is influenced by the number of fragment ions per spectrum. To assess the impact of the number of fragments per spectrum on search performance, match results obtained from the four established categories (one fragment/spectrum, two fragments/spectrum, three fragments/spectrum, and more than three fragments/spectrum) were independently analyzed. As the *ramp*-value is used as parameter to classify a match as positive or negative, we were particularly interested to detect group-specific differences in the *ramp*-value distributions. For this purpose graphs showing the number of correct matches vs. *ramp* were prepared (Figure 3). We found that irrespectively of the set *ramp*-threshold the percentage of correct matches increased with the number of fragment ions provided in a spectrum. Without considering the *ramp*-value as classifier the percentages of correct matches were for all four groups of spectra beyond 93% (Table 1). At a *ramp*-threshold of 40.0, which we usually consider as minimum value to be reached to classify a match as correct, the percentages of correct matches ranged from 80.5% for one-fragment-ion spectra to 96.0% for the more-than-three-fragment-ions spectra (Table 1). Whereas spectra containing at least three different fragment ions retrieved qualified matches in approximately 95% of cases, the percentage of qualified matches dropped below 90% for the two other categories of spectra.

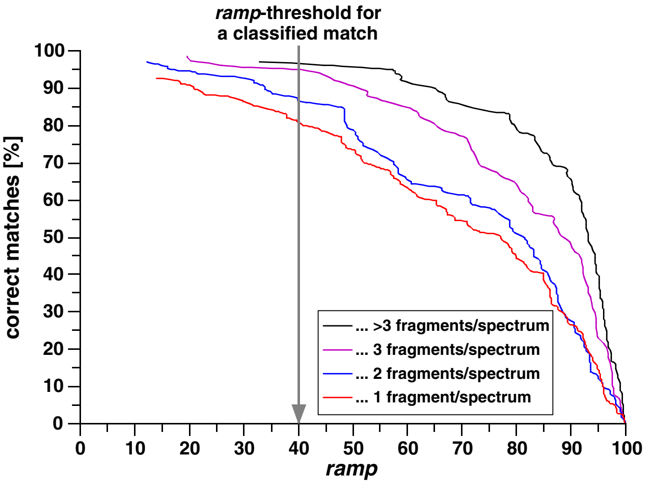


Figure 3. Statistical evaluation of the reliability of match via category-specific plots of the percentage of correct matches vs. the *ramp*-threshold.

Generally, the *ramp*-value represents the portion of the compound-specific match probability on the total sum of match-probabilities. If spectral features of a sample spectrum are shared by a large number of different reference compounds, a low *ramp*-value for the top-matching compound will be retrieved. As the probability of sharing a single fragment ion or a single precursor-to-fragment ion transition is much higher than sharing a set of spectral features, the average *ramp*-values retrieved for spectra comprising of one or two fragments are usually lower than the average *ramp*-values obtained from spectra with multiple fragment ions. Spectra with low information content are often disqualified even though the correct compound has been matched in first rank. Based on this observation we recommend searching the MSforID library with spectra that contain at least three transitions. It is important to note that searching with fewer spectral features will not automatically lead to a wrong search result. In the vast majority of cases the correct compound will be retrieved as top match. Just the likelihood of receiving a *ramp*-value below the user-defined classification threshold is somehow higher.

The impact of the number of fragment ions per spectrum on the *ramp*-value and therefore on sensitivity can be of particular importance for applications of the MSforID library on tandem-in-time instruments (i.e. IT). Generally, CID on tandem-in-time instruments tends to produce spectra with a lower number of features than CID on tandem-in-space instruments (e.g. QqQ, QqLIT, QqTOF). Thus, for unequivocal identification in cases where less than three fragment ions will be available it might be advisable to complement the MS/MS spectrum with MS^n data.

Evaluation of the selectivity of ion transitions

Spectral features (i.e. fragment ions, precursor-to-fragment ion transitions) used for the identification of compounds should represent specific identifiers. Different compounds, however, that exhibit some degree of structural similarity, tend to have fragment ions or even precursor-to-fragment ion transitions in common (e.g. morphine and hydromorphone, codeine and hydrocodone as well as doxycycline and tetracycline). Such overlapping tandem mass spectral information can be an obstacle for unequivocal compound identification. Thus, the necessity of assessing analytical specificity of transitions is well recognized for methods utilizing MS/MS detection.[27–30] Experiments aimed at evaluating the selectivity of LC/MS/MS methods during development and validation have been proposed. These include (1) analysis of several sources of blank matrix, (2) analysis of blank matrices spiked with compounds expected to be present in real samples, and (3) analysis of real samples with suspected interference(s) but without the target analyte. In this context, a fast and efficient method for identifying and sorting out putative interferences would be of help to cut down experimental efforts. The use of tandem mass spectral libraries for the proper selection of precursor-to-fragment ion transition has been proposed.[31] We additionally recommend the usage of libraries for the identification of putative interferences.[32]

To estimate the frequency of single precursor-to-fragment ion transitions shared by different compounds, all transitions extracted from literature were matched individually to the library (Figure 4). The beauty of this approach is that the library can quickly be screened for compounds having identical transitions. Sharing of a fragment ion is indicated by multiple matches: for sharing of a precursor-to-fragment ion transition multiple matches are classified by the precursor ion mass. Overall, 775 transitions out of 453 spectra were screened with the MSforID library. For this experiment only spectra containing one, two or three fragment ions were considered; 42.3% of the transitions were found to be specific. Interestingly, the portion of specific transitions was highest for the group of spectra containing only one fragment ion (54.6%). Overall, 57.7% of all transitions were found to be unspecific. In the majority of cases, however, the targeted analyte shared its ion transition with only one or two other library entries. These compounds would represent putative interferences that should be checked during LC-MS/MS method development and validation for appearance in samples analyzed as well as for coelution with the targeted analyte. All other library entries would have already been sorted out as putative sources of interference.

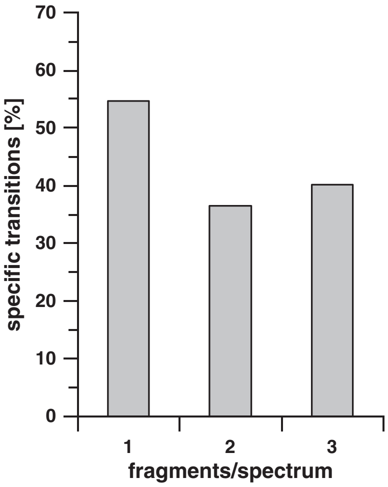


Figure 4. Category-specific frequencies of specific precursor-to-fragment ion transitions identified by matching individual transitions to the MSforID library.

In Figure 5, the frequencies of spectra containing at least one specific precursor-to-fragment ion transition in the defined categories of spectra are depicted. On average, 54.4% of spectra contained at least one specific transition. Sensitivity of the library

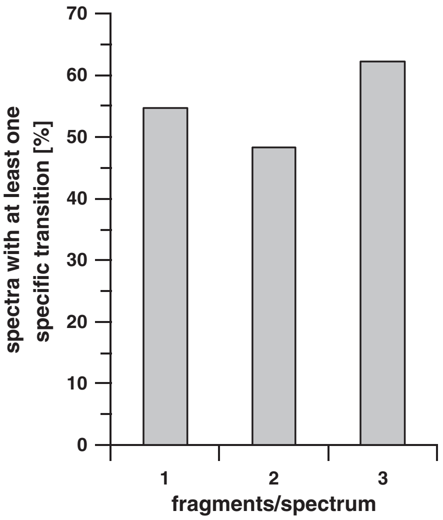


Figure 5. Category-specific frequencies of spectra containing at least one specific precursor-to-fragment ion transition identified by matching transitions to the MSforID library.

search, however, was found to be 96.4%. There are two reasons why the performance of the search algorithm exceeded the percentage of specific spectra. First, the combination of two or more unspecific features may give rise to a very specific spectrum allowing an unequivocal match to the correct reference compound. Secondly, the search algorithm does not simply count the number of matching ions. To some extend peak intensities are considered for the calculation of match probabilities. Thus, in many cases with a single dominant or view abundant transitions the corresponding compound is retrieved as top match.

Conclusions

We have extracted fragment ion mass spectra from literature. These spectra were used to evaluate the search performance of the MSforID library. Overall, 590 spectra were collected. 36 spectra were excluded from the study due to the occurrence of inconsistencies between published data and reference data, which indicates a need for more extended and more carefully performed quality control of tandem mass spectral information prior publishing. The MSforID library would contain the necessary amount of tandem mass spectral data that would allow proper selection of precursor-to-fragment ion transition as well as identification of putative interferences.

Overall 554 spectra of 291 different compounds were included in the study. Almost all common mass spectrometric instruments distributed by the five most important instrument manufacturers were covered. The percentage of correct matches was found to be 96.1% which once more proved the outstanding performance of the MSforID library in terms of transferability, robustness and accuracy.

The vast majority of spectra formed by three or more ion transitions (~95%) were found to be specific enough to enable unequivocal identification with *ramp*-values larger than 40.0. The highest percentage of unclassified search results (19.5%) was obtained with spectra comprising of only one ion, which was to a large extent attributable to the occasional sharing of transitions by different compounds.

Supporting information

Supporting information can be found in the online version of this article.

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