Elder: A Compound Identification Tool for Gas Chromatography

Mass Spectrometry Data

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Abstract

We report software entitled EIder (EI mass spectrum identifier) that provides users with eight literature reported spectrum matching algorithms for compound identification from gas chromatography mass spectrometry (GC-MS) data. EIder calculates retention index according to experimental conditions categorized by column class, column type and data type, where 9 empirical distribution functions of the absolute retention index deviation to its mean value were constructed using the National Institute of Standards and Technology (NIST) 2011 retention index database to improve the accuracy of compound identification. EIder filters compound candidates based on elementary composition and derivatization reagent, and automatically adds the molecular information of the native compound to each derivatized compound using a manually created database. When multiple samples are analyzed together, EIder performs cross-sample alignment and provides an option of using an average mass spectrum for compound identification. Furthermore, a suite of graphical user interfaces are implemented in EIder to allow users to both manually and automatically modify the identification results using experimental information at various analysis stages. Analysis of three types of GC-MS datasets indicates that the developed EIder software can improve the accuracy of compound identification.

KEY WORDS: Compound identification; GC-MS; Metabolomics; Retention index.

1. Introduction

Gas chromatography-mass spectrometry (GC-MS) is one of the most widely used analytical platforms for compound identification and quantification. A GC-MS instrument is usually equipped with electron ionization (EI) ion source, and therefore provides retention time and EI mass spectrum information for each compound. Compound identification is usually achieved by matching an experimental EI mass spectrum to EI mass spectra recorded in a reference library [1]. Retention indices (i.e., normalized retention times) can also be incorporated into identification to reduce the rate of false identifications [2].

Various algorithms have been developed to improve the accuracy of mass spectrum matching-based compound identification. Stein and Scott [1] proposed a composite measure with ratio and inner product. L_1 - and L_2 - norm approaches were also introduced to identify chemical compounds [3]. Koo, Zhang and Kim [4] developed Fourier and wavelet transform-based composite (DFT/DWT) measures. Kim, Koo, Wei and Zhang [5] found the optimal weight factors for m/z value and intensity based on cosine correlation similarity. Kim, Koo, Jeong, Wu, Shi and Zhang [6] also proposed mixture semi-partial and partial correlation-based measures. Wei, Koo, Kim and Zhang [2] developed a method to simultaneously evaluate the mass spectrum similarity and the retention index distance using an empirical mixture score function. Koo et al. [7] demonstrated that each of these algorithms has limited identification accuracy and generates different identification results. However, the software packages of these algorithms are not publicly available for the research community to test and validate for further development.

GC-MS instrument vendors also developed several software packages for compound identification, including Agilent ChemStation[®], Thermo Xcalibur[®] and LECO ChromaTOF[®].

These software packages are not open to the public and the details of the compound identification algorithms are also not disclosed. In addition, none of these software packages allows users to modify the identification results within the software.

The objective of this study was to develop a software package for compound identification from the GC-MS data, where the users can use various analytical methods to modify the analysis results before and after compound identification. We developed Elder (EI mass spectrum identifier) using MATLAB 2010b. Elder performs mass spectrum matching using literature reported eight spectrum similarity measures to improve the accuracy of compound identification. It also enables users to manually and automatically modify the analysis results to reduce the false identification rate using experimental information. The performance of Elder was tested by analyzing three experimental datasets. One dataset was GC-MS data acquired from a mixture of compound standards, while the other two datasets were GC-MS data of metabolite extracts from mouse liver and feces. Elder and sample data can be accessed at <https://sourceforge.net/projects/metaopen/files/Elder/>.

2. Materials and methods

2.1. Experimental

Three types of samples were prepared and analyzed on comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOF MS) system, including a mixture of compound standards, a metabolite extract from mouse liver, and 25 metabolite extracts from mouse feces. The mixture of compound standards was a mixture of two commercially available mixtures purchased from Sigma-Aldrich Corp. (Cat. No. A6407 and Cat.

No. A6282, St. Louis, MO, USA) that contains 42 compounds each at 500 μ M except L-cystine at 250 μ M.

To extract metabolites from mouse liver and fecal samples, each sample was first weighed and homogenized on ice. The homogenized sample was added to ice cold 80% methanol with a ratio of 50 mg of feces or liver in 1 mL of 80% methanol. The mixture was vortexed for 10 min, followed by centrifugation for 10 min at 15000 rpm. Four-hundred microliters of the supernatant was aspirated into a glass vial and dried by SpeedVac overnight at 14 °C. The dried metabolite extracts were dissolved in 40 μ L of the methoxyamine hydrochloride solution in pyridine (20 mg/mL) and vigorously vortex-mixed for 2 min.

The metabolites in the mixture of metabolite standards were derivatized using N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and the metabolites extracted from mouse liver and feces were derivatized using N-(tert-butyldimethylsilyl)-N methyltrifluoroacetamide (MTBSTFA) mixed with 1% tert-Butyldimethylchlorosilane (TBDMSCI). To derivatize samples, each sample was dissolved in 40 μ L methoxyamine hydrochloride pyridine solution (30 mg/mL) and vigorously vortex-mixed for 1 min. Methoxymation was carried out by sonicating for 20 min and incubating at 60 °C for 1 hour. Derivatization was conducted by adding 40 μ L of derivatization agent (MSTFA or MTBSTFA) to the glass vial and incubating at 60 °C for 1 hour. The stock solutions were then transferred to GC vials for analysis. The methoxymation and derivatization were prepared just before GC-MS analysis. All chemicals used for metabolite extract and derivatization were purchased from Sigma-Aldrich Corp. at St. Louis, MO, USA.

The LECO Pegasus 4D GC $\times$ GC-TOF MS instrument was equipped with an Agilent 6890 gas chromatograph and a Gerstel MPS2 auto-sampler (GERSTEL Inc., Linthicum, MD), featuring a LECO two-stage cryogenic modulator and secondary oven. The primary column was a 60 m $\times$

0.25 mm $^1d_c \times 0.25 \mu\text{m}^1d_f$, DB-5ms GC capillary column (phenyl arylene polymer virtually equivalent to a (5%-phenyl)-methylpolysiloxane). A second GC column of 1 m $\times$ 0.25 mm $^2d_c \times 0.25 \mu\text{m}^2d_f$, DB-17ms ((50%-phenyl)-methylpolysiloxane) was placed inside the secondary GC oven after the thermal modulator. Both columns were obtained from Agilent Technologies (Agilent Technologies J&W, Santa Clara, CA). The helium carrier gas (99.999% purity) flow rate was set to 2.0 mL/min at a corrected constant flow via pressure ramps. The injector temperature was set at 280 °C. The primary column temperature was programmed with an initial temperature of 60 °C for 0.5 min and then increased at 5 °C/min to 280 °C and kept for 15 min. The secondary column temperature program was set to an initial temperature of 70 °C for 0.5 min and then also increased using the same slope of temperature gradient employed in the first column to 280 °C. The thermal modulator was set to +15 °C relative to the secondary oven, and a modulation time of $P_M = 2$ s was used. The mass range was set as 29–800 m/z with an acquisition rate of 200 mass spectra per second. The ion source chamber was set at 230 °C with the transfer line temperature set to 280 °C, and the detector voltage was 1450 V with electron energy of 70 eV. The acceleration voltage was turned on after a solvent delay of 675 s. The split ratio was set at 20:1.

2.2. Analysis features of Elder

Elder has three main modules: a project meta-information module, a compound identification module and an auxiliary display module (Figure 1). The project meta-information module is designed mainly for loading input files and initializing parameters to be used for compound identification. The compound identification module calculates mass spectral similarity scores between query (experimental) spectra and reference spectra, using one or multiple spectral similarity measures. The auxiliary display module enables users either manually or automatically

to analyze data in order to reduce the rate of false identifications. These three analysis modules are seamlessly integrated through multiple graphical user interfaces (Supplementary Figures S1-S7).

2.2.1. Input/output files and reference libraries

The input files of Elder can be either raw GC-MS data in Netcdf (*.cdf) or XML (*.mzXML) formats or a text-based comma-separated values (*.csv) generated from the raw GC-MS data after spectrum deconvolution (Supplementary Table S1). Multiple files can be simultaneously uploaded to Elder for batch analysis. The output file of Elder is a text file with comma-separated values (*.csv), which is automatically saved in the same folder of the input files (Supplementary Table S2).

Elder mass spectrum library contains all EI mass spectra available in public databases, including MassBank [8] and NIST WebBook [9], where each compound is characterized by its EI mass spectrum, compound name, chemical abstract system (CAS) number, molecular formula and molecular weight. Elder also enables users to automatically build one or multiple user reference databases by adding experimental mass spectra and related information into the Elder mass spectrum library (Supplementary Figure S2). To enable users to use the commercial library, Elder can extract reference EI mass spectra from NIST main library and its replicate library and add them to the Elder library. The users are free to use one or a combination of multiple databases in the Elder library for compound identification.

While a large reference library has a greater chance to contain a compound giving rise to the experimental mass spectrum, the overall identification accuracy is reduced because there is a greater chance that multiple reference spectra are similar to an experimental mass spectrum [7]. To improve the accuracy of compound identification, Elder first allows users to remove the

reference compounds from the Elder library based on compound elementary composition. All compounds containing elements that are not specified by the users are excluded from spectrum matching (Supplementary Figure S3 (d)). Elder further allows users to exclude compounds from the reference library based on derivatization reagent used during experiment. Namely, any compounds containing chemical groups from other derivatization reagents are removed from the identification results. For example, any MSTFA derivatized compounds are removed from the identification list if MTBASTFA was used to derivatize compounds (Supplementary Figure S3 (e)).

2.2.2 Spectrum matching algorithms

A total of eight spectrum matching algorithms are implemented in Elder: Pearson's correlation, L_1 - and L_2 -norms [3], Stein and Scott's composite score [1], Fourier- and wavelet-transform-based composite score [4], weighted cosine similarity with the optimal weight factor [5], and semi-partial correlation mixture similarity scores [6] (see details in Supplementary Section 1). One or multiple algorithms can be sequentially selected for compound identification. The identification results of each chromatographic peak (using multiple identification algorithms) are automatically organized for users to assess the identification precision. It has been reported that the semi-partial correlation mixture similarity has the best identification accuracy, but it is computationally expensive. The weighted cosine method is the fastest method with comparable identification accuracy to the semi-partial correlation mixture similarity [7].

2.2.3 Retention index calculation

Retention index has been widely used to assist compound identification. If the retention times of a set of n-alkanes are available, Elder automatically calculates retention index such as Kovats and Linear retention index for each compound based on experiment conditions.

Kovats' retention index is defined as:

$$I(x) = 100 \times n + 100 \left(\frac{t_{R(x)} - t_{R(n)}}{t_{R(n+1)} - t_{R(n)}} \right) \quad (1)$$

and linear retention index is defined as:

$$I(x) = 100 \times n + 100 \left(\frac{\log(t'_{R(x)}) - \log(t'_{R(n)})}{\log(t'_{R(n+1)}) - \log(t'_{R(n)})} \right) \quad (2)$$

where $n, n + 1$ are numbers of carbon atoms in two adjacent n-alkane standards, between which a compound x was eluted from the GC column, t_R is a retention time and t'_R is adjusted retention time. Eider performs retention index matching using our previously developed algorithms [10]. Briefly, a total of 9 empirical distribution functions of the absolute retention index deviation to its mean value were constructed using the NIST 2011 retention index database information, owing to column class, column type and data type have significant effects on the retention index values acquired on capillary columns. A retention index variation window is determined from each distribution function using a user provided probability value.

2.2.4 Post identification analysis

Compounds extracted from complex samples are often derivatized to make them volatile and/or thermal stable for GC-MS analysis. A compound directly identified from a query spectrum by EI mass spectrum matching is often the derivatized version of the compound originally present in the sample. Therefore, each identified compound must be associated to its underivatized form, i.e., the native form originally present in the sample. To this end, after spectrum matching, Eider automatically adds the molecular information of the native compound (name and CAS number) to each derivatized compound using our manually created database.

After EI mass spectrum-based identification and estimation of retention index, EIder generates an interactive plot of retention index vs. retention time of all identified compounds (Figure 2), for users to further review and modify the identification results. EIder first automatically fits the retention indices and retention times of all identified compounds into a monotonic curve to show the global pattern of the retention indices of these identified compounds corresponding to their retention times. The users can then easily recognize the compounds with large distances of retention index to the fitted curve and manually remove these compounds from the identification table. This process can be repeated until the users agree with all identifications listed in the interactive plot.

EIder also automatically generates a suite of auxiliary tables and plots to assist users to modify the identification results, including a hit list of a selected chromatographic peaks, distribution of similarity scores for users to adjust the threshold of similarity scores, mass spectral comparison plot(s) containing both reference and query mass spectra, and the spectral similarity score distribution of top-50 hits of a selected chromatographic peak (Supplementary Figures S5 and S6).

After identifying compounds, EIder further enables users to explore chemical information of each identified compound by directly accessing multiple public databases, including PubChem, ChemSpider and WebBook (Supplementary Figure S7).

2. Results and Discussion

The performance of spectrum similarity measures implemented in EIder has been previously evaluated [6, 7]. Therefore, we mainly focus on the usage of EIder's auxiliary functions for purpose of improving the accuracy of compound identification. We evaluated the performance of

Elder using three GC×GC-TOF MS datasets, respectively, acquired from a mixture of compound standards, a metabolite extract from mouse liver, and 25 metabolite extracts from mouse feces. Each experimental dataset was first processed using commercial software ChromaTOF to reduce raw instrument data to a peak list, which has an EI mass spectrum, the first dimensional retention time and the second dimension retention time for every detected chromatographic peak. The deconvoluted information of each chromatographic peak was then used for compound identification using both ChromaTOF and Elder, where the NIST 2011 library was used as reference library.

While GC×GC-TOF MS data characterize each chromatographic peak by a mass spectrum and two dimensional retention times, only the mass spectrum and the first dimension retention time of each chromatographic peak were used in this study for compound identification. The current version of Elder does not have the capability to calculate the retention index from the second dimension retention time. In this study, the modulation period was set as $P_M = 2$ s. Compounds eluted from the first dimension column within a modulation period are mixed and then subjected to the second dimension column for further separation, resulting in that these compounds have the same first dimension retention time even though they may be well resolved during the first dimension separation. Compared with the one dimension GC-MS, the retention index converted from the first dimension retention time of GC×GC-TOF MS data contains a certain degree of variation caused by modulation period. While all experiments were performed on a GC×GC-TOF MS system in this study, the second dimension retention time of each chromatographic peak was not used to test the performance of Elder. Therefore, the testing data have the same information as the data acquired from one dimensional GC-MS, i.e., one dimension retention times and mass spectra.

3.1. Analysis of data acquired from the mixture of compound standards

Based on the company-provided information, the mixture of compound standards contains 42 compounds. After derivatization using MSTFA, the mixture was analyzed on GC×GC-TOF MS. The original peak list generated by ChromaTOF software consists of 243 chromatographic peaks. Figure S8 depicts a boxplot of similarity score distribution of the identified compound standards by ChromaTOF, weighted cosine and semi-partial correlation implemented in Elder, where the top hit (a compound with the highest spectrum similarity score) was considered as the identification result. It can be seen that the similarity scores of weighted cosine method and semi-partial correlation are higher than that of ChromaTOF, and the similarity scores of ChromaTOF have a much wider range than those of weight cosine and semi-partial correlation. By setting the threshold of spectral similarity ≥ 0.8 in weighted cosine and semi-partial correlation and ≥ 800 in ChromaTOF, the weighted cosine method implemented in Elder correctly identified 37 standard compounds while the semi-partial correlation in Elder identified 35 standard compounds and ChromaTOF only identified 31 standard compounds, respectively.

Table 1 lists five compounds that were the top hits for the EI mass spectra of five chromatographic peaks. Each of these was originally identified as an MTBSTFA-derivatized compounds. However, the sample was derivatized using MSTFA during the experiment and therefore, any identification results of MTBSTFA-derivatized compounds are false identifications. Elder automatically excludes all MTBSTFA-derivatized compounds using user provided derivatization method after spectrum matching. By doing so, the top hit for the chromatographic peak eluted at 26.65 min becomes “*L-Proline, 1-(trimethylsilyl)-, trimethylsilyl ester*” that is a MSTFA-derivatized compound and L-proline is a compound in the company provided list.

Elder provides two options for users to apply retention indices to filter potential false identifications. One is to calculate retention index using internal or external reference compounds and then match the retention index with library information based on experiment conditions [10]. The other is to use the interactive plot implemented in Elder. The users can either select one of these two approaches or combine them together. Figure 2(a) depicts the interactive plot that uses a regression curve to recognize compounds with large retention index variations. The retention times of all compounds identified by mass spectrum matching were first converted into retention indices. The pair of retention index and retention time of each compound is then displayed in a scatter plot. The relation between retention indices and retention times of all compounds is then fitted by a monotonic function. The users can then adjust the confidence level of curve fitting to recognize false identifications. Any compounds with retention indices outside of the confidence bands in regression analysis [11] (either above the upper boundary curve or below the lower boundary curve in Figure 2(a)) are likely to be potential false identifications. The user can examine details of these identifications using the identification table displayed on the left side of the interactive plot. For example, the users can further examine the chemical and biochemical information of an identified compound online by navigating multiple public databases, including PubChem, ChemSpider and WebBook, by clicking on an identified compound listed in the identification table. However, it should be noted that the retention index information for many of the detected compounds is not available in the current retention index library. For this reason, the interactive plot only shows the information for compounds that have retention index data in the NIST WebBook library (<http://webbook.nist.gov/>).

A high spectrum similarity threshold for mass spectral matching can induce a high rate of false negative identifications, while a low spectral similarity threshold can introduce a high rate of false positive identifications. Currently, the threshold of spectral similarity is selected by users based on their experience and confidence on the GC-MS data. EIder recommends a threshold of spectral similarity by investigating the relationship of retention index deviations between the reference values and experimental values of all identified compounds, as well as receiver operating characteristic (ROC) curve [12]. An optimal threshold of the mass spectral similarity is recommended as the spectral similarity that provides the largest true positive rate (TPR, sensitivity) and small false positive rate (FPR, 1-specificity) based on the retention index deviations of all identified compounds. For instance, the optimal similarity threshold for the mixture of compound standards is 0.81 using ROC curve, where TPR=0.81 and FPR = 0.57 shown in the Figure 3.

As shown in Figure 2(b), retention indices of compounds identified using the optimal similarity threshold of 0.81 are very close to the regression curve (the solid line). The suspected compound in the dotted circle in Figure 2(a) was removed with the suggested optimal spectral similarity threshold in the Figure 2(b). However, two compounds in the solid red circles in the Figure 2(b) are still questionable. It is up to the users to decide whether these two compounds are false identifications. It should be noted that the optimal threshold of spectrum similarity recommended by EIder tends to be conservative since the method to find the optimal similarity threshold using ROC curve is balance between TPR and FPR evenly. A practical threshold can be set to a value lower than the recommended value in order to increase TPR, that is, to obtain more true compounds in spite of high false identification.

3.2. Analysis of metabolite extract from mouse liver

To further study the performance of EIder in analyzing complex samples, we derivatized a metabolite extract of mouse liver by MTBSTFA and analyzed the derivatized sample on GC×GC-TOF MS. A total of 1984 chromatographic peaks were detected by ChromaTOF. By setting threshold of mass spectral similarity to ≥ 0.6 , EIder identified 198 compounds corresponding to 281 chromatographic peaks using weighted cosine similarity measure. The optimal threshold of mass spectral similarity recommended by EIder was 0.76. By using this EIder recommended threshold, 114 compounds were identified from 138 chromatographic peaks using weighted cosine similarity measure.

Figure S9 depicts the relationship between the number of identified compounds and the threshold of mass spectrum similarity, ranging from 0.6 to 0.95. It can be seen that an increase of mass spectrum similarity threshold decreases the numbers of derivatized, underivatized, as well as total compounds identified by mass spectrum matching. It is interesting to note that the number of identified compounds has a strong linear relationship with similarity threshold ($R^2 > 0.97$).

Table 2 shows the result of filtering compounds identified with the false derivatization reagent. A total of 8 MSTFA derivatized compounds were originally identified by mass spectrum matching. These identification results are false identifications because MTBSTFA was used as the derivatization reagent during experiment. Therefore, EIder automatically removed these compounds from the identification list based on the user provided derivatization information. By doing so, 3 chromatographic peaks were identified as MTBSTFA-derivatized compounds. EIder further automatically added the names and CAS numbers of the corresponding underivatized compounds using our in-house created database, i.e., “4,6-Dimethyl-2-thioxo-1,2-dihydro-3-pyridinecarbonitrile *tbdms*”, “2-(aminoxy)acetic acid”, and “L-Glutamic acid”. To our

knowledge, Elder is the only software package that has these derivatization-based filtering and converting processes.

3.3. Analysis of multiple samples from mouse feces

It is a common practice in metabolomics that multiple samples are analyzed in a project to increase its statistical power. Metabolite extracts from 25 mouse fecal samples were analyzed in this study on a GC×GC-TOF MS system. The instrument data were first processed using LECO's ChromaTOF software for peak picking. All peak lists generated from the 25 samples were then aligned together by minimizing the retention time variation and maximizing pairwise spectral similarity scores. A total of 99 peaks were fully aligned from the 25 peak lists; i.e., each of these 99 peaks was detected and aligned together in all 25 samples. Supplementary Information Figure S10 depicts the boxplot of relative standard deviation (RSD, %) of the first and the second dimension retention times. As expected, the second dimension retention has larger relative standard deviation than the first dimension retention time.[13]

To identify compounds from GC-MS data, every mass spectrum acquired from each sample is usually used as a query spectrum to find the best matched mass spectrum from a reference library. The compound of the best matched spectrum is then considered as the compound giving rise to the experimental mass spectrum. We termed this as the conventional approach. To reduce the variations among the mass spectra of the same compound acquired in multiple samples, we implemented an average mass spectrum approach in Elder software.

To calculate an average mass spectrum for a group of aligned mass spectra, each mass spectrum is first normalized by setting the peak height of base peak to 1000 and then scaling the peak height of the remaining peaks accordingly to form a normalized mass spectrum. The peak heights of the same fragment ion in all normalized spectra are then added, and any fragment ions

present in less than 75% of the normalized mass spectra are removed from the summed mass spectrum as random peaks. The average mass spectrum is then calculated by dividing the peak height of every fragment ion in the summed mass spectrum by the number of mass spectra.

Figure 4 depicts the performance comparison of the average spectrum approach and the conventional approach in identification of compounds from the 99 fully aligned peaks. The mean of spectral similarity scores calculated using the average spectrum approach and conventional approach are 0.885 and 0.883, respectively. In addition, the spectrum similarity scores of the average spectrum approach are much tighter and have fewer outliers. Therefore, the proposed average spectrum approach not only generates larger spectral similarity scores, but also improves the distribution of the spectrum similarity scores.

Figure 5 shows an example that the conventional approach identified three compounds from the mass spectra of a compound that were fully aligned in all 25 samples. Compound *Malic acid*, *3TBDMS derivative* was identified from 14 mass spectra, while compound *tert-butyl(dimethyl)silyl 2-([tert-butyl(dimethyl)silyl]oxy)-3-methylbut-2-enoate* was identified from 10 mass spectra and compound *2-Pentamethyldisilanyloxybutane* was identified from one mass spectrum. The first two compounds are MTBSTFA derivatized. Therefore, EIder cannot filter any of these three compounds based on either elementary composition or derivatization reagent. However, the average mass spectrum approach identifies the compound giving rise to the 25 aligned peaks as *Malic acid*, *3TBDMS derivative*, with a spectral similarity score of 0.662. Supplementary Information Figure S11 (a) and (b) are the average mass spectrum of the 25 aligned peaks and the reference mass spectrum in NIST 2011, respectively. The heaviest fragment ion detected in each of the 25 mass spectra is $m/z = 419$. The molecular weights of *Malic acid*, *3TBDMS* and *tert-butyl(dimethyl)silyl 2-([tert-butyl(dimethyl)silyl]oxy)-3-methylbut-*

2-enoate are 477 and 343, respectively. It is impossible that the compound *tert-butyl(dimethyl)silyl 2-([tert-butyl(dimethyl)silyl]oxy)-3-methylbut-2-enoate* can generate a fragment ion with $m/z = 419$. For this reason, *Malic acid, 3TBDMS* should be the true compound. In fact, Figure 5 also shows that *Malic acid, 3TBDMS* has a large mean of spectrum similarity scores and a tighter distribution than compound *tert-butyl(dimethyl)silyl 2-([tert-butyl(dimethyl)silyl]oxy)-3-methylbut-2-enoate*. This demonstrates that the cross sample alignment method implemented in Elder can correctly align the chromatographic peaks even though they may have different identifications. Furthermore, the proposed average mass spectrum approach in Elder is more robust for compound identification than the conventional approach in a study that uses multiple samples.

4. Conclusions

While multiple compound identification algorithms have been reported in the literature and several commercial software packages are available, there is no open software available in the public domain for compound identification in GC-MS data. The developed Elder is an open compound identification software package. The Elder package provides eight literature reported spectral similarity measures for comprehensive identification analysis of EI spectra acquired on GC-MS or GC×GC-TOF MS. Two public open databases, WebBook and MassBank, are used as its default reference mass spectrum library. The users have not only the option to add commercial reference library such as NIST library, but also to build in-house reference libraries. Multiple graphic user interfaces have been implemented in Elder to enable users to control false identification discovery using experimental information at different stages of analysis. Elder also automatically links each identified compound to external websites to allow users to investigate detailed compound information.

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Table 1. Derivatization-based filtering results for analysis of compound standards. The top hit was considered as the identification results. The Before Filtering columns show that MTBSTFA-derivatized compounds were identified for the five chromatographic peaks by mass spectrum matching. The After Filtering columns show the identification results by removing the MTBSTFA-derivatized compounds from the identification list.

t_R (min)	Before Filtering		After Filtering	
	Name	SC*	Name	SC*
26.65	L-Valine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	0.621	L-Proline, 1-(trimethylsilyl)-, trimethylsilyl ester	0.612
26.69	Glycine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	0.656	Glycine, N,N-bis(trimethylsilyl)-, methyl ester	0.618
28.89	Glycine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	0.620	Pentanoic acid, 4-methyl-2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	0.589
31.02	Butanoic acid, 4-[(tert-butyldimethylsilyl)amino]-, tert-butyldimethylsilyl ester	0.695	Androstan-17-one, (5 α)-	0.595
34.74	Decanedioic acid, bis(tert-butyldimethylsilyl) ester	0.634	Benzene, eicosyl-	0.582

* weighted cosine similarity score

Table 2. List of compounds identified from mass spectra of eight chromatographic peaks with and without applying derivatization-based filtering. The threshold of similarity score was set as 0.8.

t_R (min)	Before filtering derivatized approach	After filtering derivatized approach
13.53	Ethanamine, N,N-dimethyl-2- [(trimethylsilyl)oxy]-	2-Amino-2-methyl-1-propanol, Isobutanolamin
17.33	Propanoic acid, 2-(methoxyimino)-, trimethylsilyl ester	l-Norvaline, N-(2-methoxyethoxycarbonyl)-, octyl ester
19.13	Tris(trimethylsilyl)borate	4,6-Dimethyl-2-thioxo-1,2-dihydro-3- pyridinecarbonitrile tbdms
25.10	Tris(trimethylsilyl)carbamate	2-(aminooxy)acetic acid
25.33	Ethanedioic acid, bis(trimethylsilyl) ester	1-Pentamethyldisilyloxy-10-undecene
28.20	1-(2-Methoxyethoxy)-2-methyl-2-propanol, trimethylsilyl ether	Silane, butoxytrimethyl-
39.77	L-Glutamic acid, N-(tert- butyldimethylsilyl)-, bis(tert- butyldimethylsilyl) ester	L-Glutamic acid
46.13	Arachidonic acid, trimethylsilyl ester	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-

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452 **Figure 1.** Workflow of compound identification in software Elder.

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Figure 2. Auxiliary figure of retention index versus retention time corresponding to threshold of similarity scores 0.6 in (a) and 0.81 in (b). The dotted and solid circles indicate compounds that are outside of 95% confidence bands in regression analysis.

Figure 3. Receiver operating characteristic (ROC) curve and the EIder recommended optimal threshold of similarity score. The red circle represents estimated optimal threshold, where the suggested optimal similarity threshold is 0.81 with TPR (sensitivity) = 0.81 and FPR (1-specificity) = 0.57.

Figure 4. Boxplot of spectral similarity scores of the 99 fully aligned compounds calculated using average mass spectrum approach and conventional pairwise comparison.

Figure 5. Boxplot of similarity scores of each unique compound name in the example aligned peak.