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Open-source Python module to automate GC-MS data analysis developed in the context of bio-oil analyses

Matteo Pecchi *[ab] and Jillian L. Goldfarb [ab]

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GC-MS (Gas Chromatography-Mass Spectrometry) is widely used to measure the composition of biofuels and complex organic mixtures. However, the proprietary GC-MS software associated with each instrument is often clunky and cannot quantify compounds based on similarity indices. Beyond slowing individual research group's efforts, the lack of universal free software to automatically process GC-MS data hampers field-wide efforts to improve bio-oil processes as data are often not comparable across research groups. We developed "gcms_data_analysis," an open-source Python tool that automatically: (1) handles multiple GCMS semi-quantitative data tables (whether derivatized or not), (2) builds a database of all identified compounds and relevant properties using PubChemPy, (3) splits each compound into its functional groups using a published fragmentation algorithm, (4) applies calibrations and/or semi-calibration using Tanimoto and molecular weight similarities, and (5) produces multiple different reports, including one based on functional group mass fractions in the samples. The module is available on PyPI (https://pypi.org/project/gcms-data-analysis/) and on GitHub (https://github.com/mpecchi/gcms_data_analysis).

1 Introduction

Gas Chromatography-Mass Spectrometry (GC-MS) is routinely used to identify and quantify organic mixtures. In particular, GC-MS analysis of liquid biofuels enables assessment of the composition and therefore quality and value of these sustainable fuels.[1–3] The detailed compositional analysis from GC-MS of bio-oil can reveal the chemical reactions underpinning biofuel production processes, thus enabling the optimization of process conditions based on feedstock composition.[4–6]

Performing qualitative GC-MS analysis of bio-oil samples is relatively straightforward; bio-oil is routinely extracted using an organic solvent and compounds are identified by spectral library matches.[7–9] In theory, the quantification of compounds *via* GC-MS is rather simple; chromatogram area is linearly related to the concentration of each analyte. In practice, quantifying the vast numbers of compounds detected in heterogeneous bio-oils requires the construction of a large calibration dataset.[10–12] Depending on the GC-MS column, compounds present, and their concentrations, derivatization may be necessary to improve identification, reproducibility and therefore final quantification of species present.[6,13,14] Derivatization requires its own calibration to quantify derivatized peaks, sometimes at different operating conditions than the non-derivatized analysis.

These experimental challenges are compounded by the handling and analysis of the large amount of data that GC-MS analyses produce. A single bio-oil sample can contain hundreds of compounds.[15,16] A typical experimental campaign involves dozens of samples (hopefully with at least a modicum of replication). Thus, the analysis of such datasets is onerous; the application of a calibration curve to quantify each individual component in a bio-oil, and the translation from derivatized results to the original bio-oil components, both add downstream computational tasks. Manual handling of such large datasets introduces the risk of human error.

In general, most GC-MS instruments provide solutions for the identification of compounds through fragmentation pattern matching by means of proprietary software, and several open-source options are available for the task. One example is PyMS[17] and its spin-off project, PyMassSpec.[18] Using these open-source tools or the proprietary software available on GC-MS sample it is possible, for each sample, to produce a semi-quantitative table with the identified compounds and their measured chromatogram area, which indicates the intensity of the signal for each compound. If a user has developed a calibration curve for a particular compound, the instrument's software will (usually) report the concentration of the component in the sample vial. For derivatized samples, the derivatized version of the compound is usually returned (since the derivatized molecule enters the machine), which requires an

[a]*Department of Biological & Environmental Engineering, Cornell University, Ithaca, NY 14853, USA. E-mail: mp933@cornell.edu*
[b]*Smith School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY 14853, USA*

additional step to translate the derivatized versions into their non-derivatized (original) compound. Most instrument companies' proprietary software handle calibrations, but their use can be counterintuitive and/or difficult to implement in additional automatic data analysis routines. Both from our own experience and conversations with researchers around the world, calibrations are often applied manually by researchers, especially when a semi-calibration mode is adopted to estimate the concentration of non-calibrated compounds based on similar compounds' calibration curves.

Another bottleneck in the analysis of GC-MS data is the classification of identified compounds based on their functional group(s). This is especially useful for heterogeneous mixtures such as bio-oils where aggregated compositional results (*e.g.*, the fraction of compounds having a given functional group present in each sample) can be used to derive mechanistic conclusions.[5,7,9,19] While performing these tasks manually is possible and has led to valuable results, automation could save time and minimize human error, while also increasing transparency in the methodological approach, specifically in how functional groups are attributed to each compound. Instruments' proprietary software is not always user-friendly or intuitive, and often requires extensive training, especially when used for quantitation of non-calibrated compounds. Such proprietary software does not allow for automation of the full GC-MS data analysis pipeline, usually limiting the automation at the single sample analysis. As such, a new tool for researchers that is open-access and incorporates multiple automated analysis pathways could save researchers' time and promote a higher level of standard best practices across GC-MS users, especially in the biofuels field.

To address this need, we designed an open-source Python tool to fully automate the handling of multiple GC-MS datasets simultaneously (a note, our tool does not perform the analysis of raw GC-MS spectral data, for that, please refer to [18]). The tool relies on the Python package *PubChemPy*[20] to access the PubChem website[21] and build its own database with all identified chemicals and their relevant chemical properties. It also performs functional group fragmentation using each compound's SMILES[22] retrieved from PubChem, employing the automatic fragmentation algorithm developed by ref. 23 to split each molecule into its functional groups and then assign to each group its mass fraction. Functional groups can also be specified by the user using their SMARTS codes.[24] The code can apply calibrations for quantifying components present in a sample, with an semi-calibration option based on Tanimoto similarity with tunable thresholds.[25,26] For derivatized samples, the procedure is unchanged except that the non-derivatized form of each identified compound is used to retrieve chemical information so that non-derivatized and derivatized samples can be directly compared. The tool groups replicate "files" (intended for single analyses) into the same "sample" (intended for the average of multiple replicates of single analyses of the same sample) to evaluate reproducibility; the user only needs to ensure the proper naming convention is adopted.

Besides producing single sample (or individual files) reports, the tool produces comprehensive reports that include all compounds across a series of samples (files) as well as aggregated reports for all samples (files) based on functional group mass fractions. The code also provides plotting functions for the aggregated reports to visualize the results and enable a preliminary investigation of their statistical significance. The module can be easily installed from PyPI (**https://pypi.org/project/gcms-data-analysis/**) using pip or downloaded from GitHub (**https://github.com/mpecchi/gcms_data_analysis**). A simplified example is available on GitHub as a tutorial for new users (with an artificial dataset that was simplified to make testing and maintenance feasible).

In this paper, we demonstrate the tool's capability using a set of three real non-derivatized and derivatized bio-oil samples (analyzed in triplicate); the real data used in this manuscript (found in the subfolder "RCSdata" in the GitHub repository) differ from the example case on GitHub, which is simplified to ensure that no user accidentally employs our calibration curves for their instruments.

2 Algorithm

This section describes the conceptual principles used to design the algorithm. For details about function, naming conventions, and detailed documentation, please refer to the GitHub repository (**https://github.com/mpecchi/gcms_data_analysis**). For examples of file formatting, the reader should refer directly to the "RCS/data" folder in said GitHub repository.

An overview of the algorithm is provided in Table 1. The algorithm on the left is divided into steps that perform sequential tasks, highlighting the inputs and outputs the code requires or produces in each part. Main libraries and functions used by the algorithm are described in the relevant sections. Details of each part are given in the following subsections.

2.1 Part 1: load (or create) input files

The user needs to firstly specify the path to the project folder where the input files are located and where the output files will be created.

The algorithm can then load (or create) the following input files:

(1) "files_info.xlsx" file: a table that contains, for each "file" (intended as a single experiment or replicate), the information provided below as columns.

- "filename": the name of the GC-MS semi-quantitative data table file (the report of the GC-MS analysis of the sample that contains the identified compounds' names and their peak area).
- "derivatized": Boolean indicating whether the sample was derivatized or not.
- "dilution_factor": a int or float that indicates the dilution factor of the sample (in *x*-folds, *e.g.*, a 10-fold dilution would only report "10"). If no calibration is used, the only available comparison is between absolute area for the same identified compound across samples (since area is directly proportional to concentration but with an unknown factor for each compound). If different samples are obtained with the same methodology but need to be analyzed at different dilutions (for example to

Table 1 Overview of the algorithm used in the "gcms_data_analysis" module

Part	Action	Input	Output	Methods
1	Load input files	"files_info.xlsx" Files (GC results), calibration files "compounds_properties"	Data is stored in the project class	Pandas
2	Create compounds properties (if not available)	"classifications_codes_fractions"	"compounds_properties"	pubchempy, rdikit, frag. Algoritm[23]
3	Apply calibration to files	Files, calibrations	Updated files with concentrations	Pandas, numpy
4	Create samples from files	Files	Samples (average), samples (standard deviation)	Pandas, numpy
5	Create multi-file (or sample) reports	Files (or samples)	Report: one parameter (conc, area) for all files or samples	Pandas
6	Create multi-file (or sample) aggregated reports (by functional groups)	Files (or samples), compounds properties with functional group splitting	Report: one aggregated parameter (conc, area) for all files or samples	Pandas
7	Plot reports or aggregated reports	Report or aggregated report	Figures	Matplotlib, pandas

protect the GC-MS detector from saturation), specifying such dilution factor allows the code to compute the "area_if_undiluted" (measured area multiplied by the dilution factor) of compounds in each sample that can then be used to compare "relative concentrations" (not absolute ones). If unspecified, the default value is 1. The same approach is used to compute "conc_vial_if_undiluted_mg_L″ if a calibration is available.

• "calibration_file": The name of the Calibration file to use for the sample, if available (in the example "calibration" and "deriv_calibration". This is used to load the right calibration file to compute concentration for the sample.

• "total_sample_conc_in_vial_mg_L": The measured sample concentration in mg_{sample} per $L_{solvent}$ where mg_{sample} indicates the mg of undiluted sample (without any solvent) in the GC-MS vial (must be obtained either gravimetrically or *via* mass balances). If a calibration is available, the code computes the concentration of each identified compound in the sample. The sum of the identified fractions gives the overall sample fraction; it is compared with the measured sample concentration to calculate the detected sample fraction, an indication of how much of the sample was identifiable through GC-MS analysis. If not available, default is set to 1.

• "sample_yield_on_feedstock_basis_fr": If the sample is produced from a feedstock with a known yield, the user can include such yield (ex.: the bio-oil yield on feedstock basis). This is used to compute single compound yields on feedstock basis. If no yield is specified, 1 is used as default and the results can be ignored.

(2) One semi-quantitative data table for each sample, usually as given by the GC-MS. Files must be named "samplename_replicatenumber" (with samplename a string, and replicatenumber an integer). To make this software agnostic to the type of GC-MS used, the user must specify the number of rows at the top of the file that must be skipped (typically if the file contains a summary of the GCMS method before the peak table) and the exact name for the columns that, for each identified peak, contain information about:

• Retention time of peaks.

• Names of the identified compounds (not necessarily in the IUPAC form).

• Area of each peak.

• Height of each peak.

(3) Calibration files, each sample can refer to a different calibration file by specifying it in the "files_info" table, that contains:

• The name of each calibrated compound.

• The concentrations (in our example, we use ppm on a mass per volume basis, mg L^{-1}) and of intensity (area) for each calibrated compound. Columns must be named "PPM #" and "Area #" (# = incremental natural number) for each investigated concentration. The calculated results will match the provided concentrations (ppm on mass or volume basis, or on mass/volume, depending on the users' calibration units).

(4) "classification_codes_fractions.xlsx": the list of Functional Group Classification Code Fractions: a list of names of the functional group, the SMARTS codes (SMILES arbitrary target specification) associated, and their molecular weight. This information is used to automatically fragment each identified compound into its functional groups also calculating the mass fraction of said functional groups in the identified compound. The file can be modified by the user depending on the functional groups that are of interest (details are given in Section 2.2.1).

(5) "compounds_properties.xlsx" or "deriv_compounds_properties.xlsx": see next subsection.

2.2 Part 2: build compound's property file

The code can create (or load, if available) a database that contains the properties of all compounds identified in all files and in all calibrations, the "compounds_properties". These files contain information about IUPAC name, molecular formula,

canonical SMILES, molecular weight, elemental composition, *etc.* for every compound. Data are retrieved from the PubChem website[21] using PubChemPy,[20] a Python package to automatically access the PubChem database. PubChemPy needs internet access to perform its operations and can retrieve the full set of properties of a chemical compound using any of its synonyms.

If a file is derivatized, identified compounds in the semi-quantitative data table should be in their derivatized form (ex.: "phenol, TMS derivative"); the non-derivatized form is obtained splitting the name at the last comma (ex.: "phenol"). Then, the code queries the PubChem database through PubChemPy using the non-derivatized form to retrieve chemical information. If at least one sample is derivatized, a second database (named "deriv_compounds_properties") with only compounds found in derivatized samples is created or loaded. If a derivatized calibration file is provided (in the example "deriv_calibration"), compound names must be given in their underivatized form.

After these chemical properties are retrieved, each compound is categorized by its functional groups and corresponding their mass fraction (see Section 2.2.1 for details). This operation relies on an heuristic algorithm developed in Python;[23] its code is available at ref. 27. It also requires a list of the functional groups to be searched, the "classification_codes_fractions.xlsx" file, specified in Section 2. In the present work, the simple fragmentation option of the heuristic algorithm is used.

2.2.1 Automatic fragmentation and aggregation by functional group. Generally, when discussing chemical differences among bio-oil samples (*e.g.* to assess variation in conversion mechanisms when different process parameters are employed), describing the differences in terms of single chemical compounds across samples may not be feasible due to the large number of compounds at low concentrations. A typical strategy is to aggregate compounds based on chemical families, usually by functional group, either by count or concentration.[5,7,9,28] This requires aggregating compound concentrations (or areas, though this is only a surrogate for concentrations and does not allow for comparisons across laboratories as each instrument and its parameters impact relative concentration to area ratios) based on their function group(s). A first approach (usually performed manually) requires attributing one functional group to each molecule and then calculating the cumulative aggregated area or concentration for all compounds that contain the same functional group. This approach neglects all functional groups after the first one (belonging to the same compound) and usually relies on the IUPAC hierarchy to select the "most important" functional group.[5,7,9] However, the IUPAC hierarchy was designed with naming purposes only and may not reflect the chemical importance of said groups,[29] especially as this "importance" may be relative to the type of sample being analyzed.

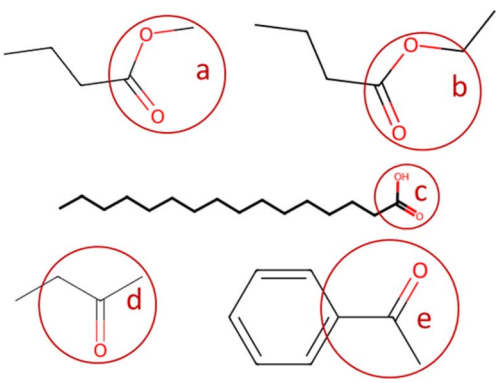
An alternative approach (though extremely time consuming if performed manually) is to calculate the mass fraction of each functional group present in the compound and use these mass fractions to split the area or the concentration of each molecule into weighted averages of the different functional groups present, and then compute the aggregated value for all compounds present in the sample. This has the advantage of fully accounting for all functional groups in each sample. This approach uses eqn (1) to compute the aggregated concentration of each functional group (C_{fg}) in the sample, obtained as the sum over all n identified compounds in the sample of the concentration of the compound (C_i) multiplied by the mass fraction of the considered functional group in the compound itself ($mf_{fg,i}$). This equally applies to area, concentration, and yield, though of course only concentration and yield data would be comparable across different laboratories.

$$C_{fg} = \sum_{i=1}^{n} C_i \times mf_{fg,i} \quad (1)$$

This latter approach is automated in the present tool. The fragmentation into functional groups relies on the fragmentation algorithm developed by ref. 23, available at ref. 27. This

Table 2 Example of SMARTS codes for functional group identification and their mass fractions. Figure codes refer to the molecules on the side of the table

Functional group code	SMARTS code	Mass fraction	Figure code
Ester	[CH_0](=O)O[CH_3]	59.044	a
Ester_1	[CH_0](=O)O[CH_2]	58.036	b
Carboxyl	[CH_0](=O)O	45.017	c
Ketone	[CH_2]C(=O)[CH_3]	58.08	d
Ketone_1	[CH_3]C(=O)[C]	55.056	e



heuristic algorithm performs the automatic fragmentation of molecules (relying on their canonical SMILES) into subunits (patterns), specified by their SMARTS code, (SMILES arbitrary target specification). This is done *via* heuristic group prioritization; heuristically determined descriptors are used to sort the patterns of the fragmentation scheme, parent–child group prioritization (patterns contained in other larger patterns are searched last), and adjacent group search (subsequent matches must be adjacent to the already fragmented structure to avoid incomplete group assignment). The algorithm provides two options: a simple fragmentation and a complete fragmentation.

In the present paper, the code loads the "classification_codes_fractions.xlsx" file described in Section 2.1, that contains a list of names of the functional group, their SMARTS codes, and their molecular weights. The smarts codes are used by the automatic fragmentation algorithm developed by ref. 23 to count the instances of each functional group, and their molecular weights are used to compute their mass fractions. A brief example of functional group SMARTS and mass fractions is provided in Table 2; when more variants (ex. ester and ester_1) of the same functional group are available (with slightly different molecular mass, mostly due to H atoms), the code considers them to be of the same functional group (they are named as the general name of the functional group followed by "_#", with # = incremental natural number). An example of automatic fragmentation for selected chemical compounds with different functional groups is shown in Table 3.

2.3 Part 3: apply calibrations to files

For each file specified in the "files_info" table, its GC-MS semi-quantitative data table is loaded, together with the relevant

Table 3 Example of functional group mass fraction for representative identified compounds obtained using the automatic fragmentation algorithm. Results are in (wt%)

IUPAC name	C-aliph	C-arom	N-arom	O-arom	Alcohol	Aldehyde	Carboxyl	Ester	Ether	Ketone
Tetradecanoic acid	0.80	—	—	—	—	—	0.20	—	—	—
Benzene-1,4-diol	—	0.69	—	—	0.31	—	—	—	—	—
3-Hydroxybenzaldehyde	—	0.62	—	—	0.14	0.24	—	—	—	—
Ethenyl hexanoate	0.60	—	—	—	—	—	—	0.40	—	—
1-(3-Hydroxyphenyl)ethanone	—	0.47	—	—	0.12	—	—	—	—	0.40
4-Butoxyphenol	0.26	0.39	—	—	0.10	—	—	—	0.25	—
2-Methyl-1-benzofuran-5-ol	0.10	0.68	—	0.11	0.11	—	—	—	—	—
Phenol	—	0.82	—	—	0.18	—	—	—	—	—
2-Methylpyrazine	0.16	0.54	0.30	—	—	—	—	—	—	—
Benzoic acid	—	0.63	—	—	—	—	0.37	—	—	—

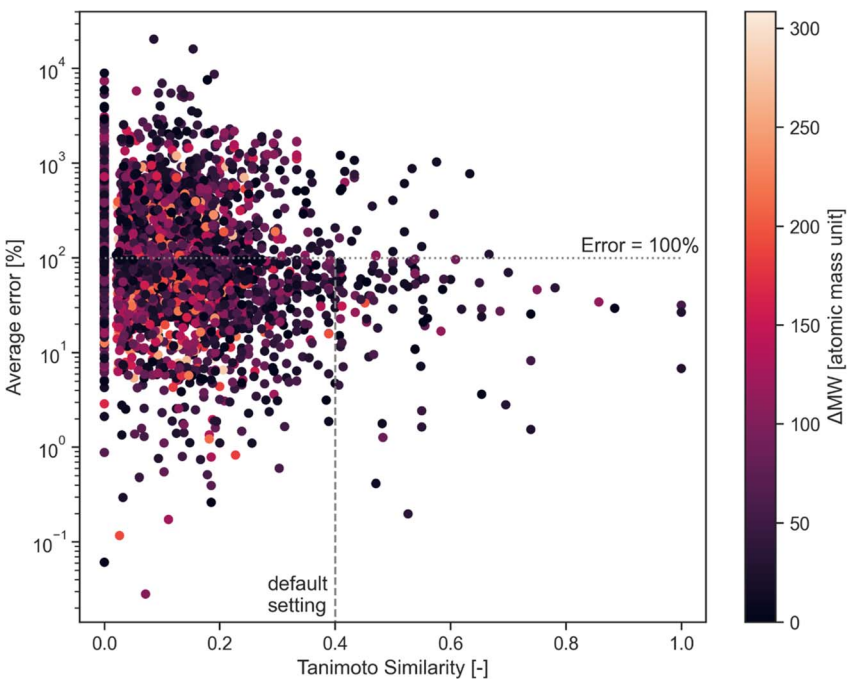


Fig. 1 Average error between calibration curves of combination of compounds as a function of their Tanimoto similarity. The molecular weight difference of the compounds is reported in the colormap. The horizontal like indicates 100% error, the vertical line reports the default similarity threshold adopted in the code.

calibration file (also specified in the "files_info" table). The semi-quantitative table is updated – the original information is maintained, except all unidentified peaks are grouped under one peak – with all the results calculated by the algorithm.

The updated semi-quantitative table for files contain, for each identified compound:

• Its IUPAC name, molecular weight, and functional group mass fractions.

• The original peak height and peak area as measured by the GC-MS.

• The computed "area_if_undiluted" (using the dilution factor, if provided, see Section 2.1).

• The computed concentrations ("conc_vial _mg_L" and "conc_vial_if_undiluted_mg_L") obtained by applying the calibration to the measured areas. This step requires a calibration file, if not available, *nan* values (not a number) are returned instead. The code also has the possibility of using a semi-quantitative calibration based on Tanimoto and molecular weight similarity to apply a similar compound's calibration to compounds that are not calibrated; details are provided in Section 2.3.1.

• The "compound_used_for_calibration" column contains information about the calibration: if a calibration for the compound was available, the column indicates "self" (meaning the compound was calibrated using "itself"). If a calibration based on a similarity index is applied, the name of the compound used as calibration surrogate, its similarity index with the compound that is semi-calibrated, and their difference in molecular weight are instead reported in the column.

• The computed mass fraction of compounds on a sample basis ("fraction_of_sample_fr"). The "fraction_of_sample_fr" is obtained by diving the concentration of the compound in the GC-MS vial (obtained by applying the calibration) by the "total_sample_conc_in_vial_mg_L" (that must be computed by the operator and included in the "files_info" table, see Section 2.1). This requires a calibration file and the sample concentration in the vial. The sum of all compound fractions gives the total amount of sample that can be identified by GC-MS.

• The computed mass fraction of compounds on a feedstock basis ("fraction_of_feedstock_fr"). The yield of the compound on feedstock basis (if provided). This requires a calibration file, the net amount of sample in the vial, and the yield of sample on feedstock basis ("sample_yield_on_feedstock_basis_fr").

2.3.1 Semi-calibration based on Tanimoto and molecular weight similarity. Since calibrating with pure standards for each identified compound in complex samples such as heterogeneous bio-oil is likely infeasible due to the large number of identified compounds, most bio-oil discussions rely on relative concentrations based on identified peak areas. In other cases, GC-MS are calibrated for some of the identified compounds, and authors adopt strategies to infer calibrations for compounds without existing calibration.[30–33] In general, these methods are often manually implemented and revolve around the use of some sort of nearest neighbor method, where for each compound without a calibration curve, the calibration available for the "closest" compound is used instead. The definition of closest varies by research group and leads to different accuracies. The simplest approach is to select the closest compound in terms of molecular weight, but this approach neglects the chemical nature of each molecule and can lead to large errors. A more sophisticated approach, so far unexplored in the biofuel GC-MS literature but common in cheminformatics,[34] is to use molecular fingerprints (encodings of the molecular structure) to compute similarity indices to select the most similar calibrated compound. A popular choice in the cheminformatics literature is the Tanimoto similarity index.[25,26]

The present code allows for semi-calibration (if the option is selected); for compounds without a calibration curve, their Tanimoto similarity with all calibrated compounds is evaluated. The calibrated compound with the highest similarity is selected (if more compounds share the same similarity, as happens for compounds of the same class, the compound with the closest molecular weight is selected). The Python package *rdkit*[35] is used to convert canonical SMILES into molecular fingerprints and to compute the Tanimoto similarity among compounds. The user can change the minimum similarity threshold and maximum molecular weight difference to apply the semi-calibration to each non-calibrated compound. If no calibrated compound meets the criteria or if the user sets the semi-calibration option to false, the compounds for which no calibration compounds are available will have all concentrations set to not-a-number (though its areas are persevered). An example of similarity table for tetradecanoic acid as compared to several compounds is provided in Table 4.

2.3.2 Accuracy of the semi-calibration approach using Tanimoto similarity. The error associated with the use of the semi-calibration approach (for example, assuming use of the calibration curve of compound c1 to estimate the concentration of compound c2) can be evaluated as the average error between the calibration curves of those two compounds, if both c1 and c2 calibration curves are known. This error equals the error that would come from using the calibration of c1 for estimating the

Table 4 Example of Tanimoto similarity and molecular weight difference between tetradecanoic acid (SMILES: CCCCCCCCCCCCCCC(=O)O) and several compounds

Compound	SMILES	Tanimoto similarity	Molecular weight diff. [g mol^{-1}]
Hexadecanoic acid	CCCCCCCCCCCCCCCC(=O)O	1	28.05
Octadecanoic acid	CCCCCCCCCCCCCCCCCC(=O)O	1	56.13
(*z*)-Octadec-9-enoic acid	CCCCCCCCC=CCCCCCCCC(=O)O	0.74	54.13
(9*z*,12*z*)-Octadeca-9,12-dienoic acid	CCCCCC=CCC=CCCCCCCCC(=O)O	0.65	52.03
Oxacycloheptadecan-2-one	C1CCCCCCCC(=O)OCCCCCCC1	0.03	26.04

concentration of c2, should c2 not be available. The average error can be computed using eqn (2), where Cal_{c1} and Cal_{c2} are the calibration curves for c1 and c2 obtained by the linear interpolation of runs at known concentrations.

$$\text{Average error } [\%] = \left|\overline{Cal_{c1} - Cal_{c2}/Cal_{c1}}\right| \times 100 \qquad (2)$$

This error has been assessed for all combinations of calibrated compounds in our calibration dataset in our GC-MS (details are provided in Section 3); this dataset is not the one provided in the GitHub repository in the "example" subdirectory as its size is not appropriate for a tutorial. The dataset is included in the RCSdata subfolder in the GitHub repo. The data set comprises 89 compounds for which a ≥4 points calibration (up to 6 points) is available in the form of mg L^{-1} *vs.* detected area; this results in 3827 combinations. For each combination of compounds, the Tanimoto similarity and the molecular weight difference is also computed. Fig. 1 plots the average error as a function of the Tanimoto similarity of compounds and their molecular weight difference. The error decreases with the increasing Tanimoto similarity, while there seems to be no marked effect of molecular weight difference. Selecting a Tanimoto similarity threshold of 0.4 minimizes the risk of errors that are above one order of magnitude, while a similarity of 0.7 avoids this almost entirely (at least in our dataset). The percentage error threshold of 100% may seem unreasonably high, however the alternative would be to simply ignore all the compounds for which no calibration is available which also implies a large data loss. The selection of a Tanimoto similarity threshold that avoids unrealistic overestimation of concentrations (underestimations are less of a concern, since the alternative would be to ignore the compound entirely) improves the quality of GC-MS results. A similarity threshold of 0.4 was arbitrarily chosen as the default threshold in the code based on these results, with a molecular weight difference a threshold of 100 atomic mass units set as the default.

The user is encouraged to run a similar analysis on their calibration database to assess what thresholds are most appropriate for their use case.

2.4 Create samples from files (replicates)

After each file is processed, those files that belong to the same sample are used to compute average values and standard deviations for each sample (in the example, files "A_1", "A_2", and "A_3" are replicates of sample "A").

2.5 Part 4: build multi-file and sample reports

The code can create multi-file and multi-sample report tables with a list of all identified compounds as the rows, and the results for each sample and for each parameter (area, concentrations, and yield) as the columns.

2.6 Part 6: build multi-file and sample aggregated reports

The tool can also produce aggregated reports by functional group for each sample. Aggregated reports have samples as rows

Table 5 Selected rows and columns for the updated sample report that describes the GC-MS analysis of sample A_1. Areas are in total ion counts, concentrations are in mg L^{-1}, fractions are g g^{-1}

A_1	iupac_name	retention_time	Area	area_if_undiluted	conc_vial_if_undiluted_mg_L	fraction_of_sample_fr	fraction_of_feedstock_fr	compound_used_for_calibration
Unidentified	Unidentified	6.025	373 897	934 7425				n.a.
Tetradecanoic acid	Tetradecanoic acid	36.163	44 389	1 109 725	589.5376	0.04211	0.021055	Self
Oxacycloheptadecan-2-one	Oxacycloheptadecan-2-one	40.052	15 068	376 700				n.a.
n-Hexadecanoic acid	Hexadecanoic acid	40.492	1 878 180	46 954 500	1651.359	0.117954	0.058977	Self
9,12-Octadecadienoic acid (*z*,*z*)-	(9*z*,12*z*)-Octadeca-9,12-dienoic acid	43.847	1 456 119	36 402 975	3279.7	0.234264	0.117132	Self
Oleic acid	(*z*)-Octadec-9-enoic acid	43.986	6 379 752	1.59 × 108	2840.463	0.20289	0.101445	(*e*)-Octadec-9-enoic acid (sim = 1.0; dwt = 0)
Octadecanoic acid	Octadecanoic acid	44.402	1 635 947	4 089 8675	1648.929	0.117781	0.05889	Self
9-Octadecenamide, (*z*)-	(*z*)-Octadec-9-enamide	44.733	62 240	1 556 000	635.495	0.045393	0.022696	(*e*)-Octadec-9-enoic acid (sim = 0.7; dwt = 1)
13-Docosenamide, (*z*)-	(*z*)-Docos-13-enamide	47.947	21 557	538 925	621.2956	0.044378	0.022189	(*e*)-Octadec-9-enoic acid (sim = 0.7; dwt = 55)

and aggregated results by functional group as columns, obtained as the weighted sum of each parameter, (area, concentration) where the weights are the functional group mass fractions of each compound. Details of the aggregation by functional group procedure are given in Section 2.2.1.

2.7 Part 7: saving and displaying results as plots

The module automatically saves all tabulated outputs by default in dedicated subfolders in a "output" subdirectory. It also provides a straightforward way to directly plot the content of multi-file and multi-sample reports and aggregated reports. With files (as opposed to samples), plots do not show error bars (as each file is analyzed alone). For sample, each value is reported as the average and standard deviation of the files that constitute the sample. For details, we refer the reader to the documentation and to the example in the GitHub repository.

3 Test cases of three bio-oil samples

This case study describes real results for 3 bio-oil samples that belong to a larger experimental campaign that will be discussed in a separate publication. The context of the experiments and the implications of the results are only discussed here to

Table 6 Selected rows for the "conc_vial_mg_L" report with all analyzed files

conc_vial_mg_L	A_1	A_2	A_3	Ader_1	Ader_2	Ader_3	B_1	B_2	B_3
4-Oxopentanoic acid	0	0	0	0	0	0	683.4494	776.1829	768.8858
(9*z*,12*z*)-Octadeca-9,12-dienoic acid	131.188	125.3808	86.76279	31.36777	36.81299	27.92726	0	0	0
(*z*)-Octadec-9-enoic acid	113.6185	113.8273	84.92259	21.66908	24.27344	19.93824	0	0	0
Furan-2-carbaldehyde	0	0	0	0	0	0	72.39856	0	0
Hexadecanoic acid	66.05436	61.11673	47.13392	27.62319	27.3815	19.56924	29.1569	31.45626	32.20709
Octadecanoic acid	65.95717	59.31563	53.60064	14.2637	17.55913	11.2483	35.62859	37.75122	37.75725
3-Methylcyclopentane-1,2-dione	0	0	0	0	0	0	36.33573	7.318511	7.234667
5-(Hydroxymethyl)furan-2-carbaldehyde	0	0	0	0	0	0	31.55445	34.74618	35.66275
Ethenyl hexanoate	0	0	0	0	0	0	33.06538	0	0
5-Methylfuran-2-carbaldehyde	0	0	0	0	0	0	25.57821	27.62023	27.29056

Table 7 Selected rows for the average and deviation "conc_vial_mg_L" reports with all analyzed samples

	A	Ader	B	A	Ader	B
conc_vial_mg_L	Average			Standard deviation		
4-Oxopentanoic acid	0	0	742.8394			51.56245
(9*z*,12*z*)-Octadeca-9,12-dienoic acid	114.4439	32.03601	0	24.14771	4.480395	
(*z*)-Octadec-9-enoic acid	104.1228	21.96026	0	16.6282	2.182219	
Octadecanoic acid	59.62448	14.35704	37.04568	6.184055	3.156452	1.227244
Hexadecanoic acid	58.10167	24.85798	30.94008	9.813955	4.581773	1.589258
5-(Hydroxymethyl)furan-2-carbaldehyde	0	0	33.98779			2.156596
Tridecane-5,8-dione	0	0	26.88192			
5-Methylfuran-2-carbaldehyde	0	0	26.82967			1.096256
Furan-2-carbaldehyde	0	0	24.13285			41.79933

Table 8 Aggregated results (sample fraction [g g^{-1}]) for each sample and functional group

	A	Ader	B	A	Ader	B
conc_vial_mg_L	Average			Standard deviation		
C-aliph	0.615577	0.827408	0.078768	0.136911	0.118395	0.012261
Ketone	0	0	0.168349	0	0	0.016044
Carboxyl	0.116397	0.140476	0.113552	0.016592	0.021211	0.007693
C-arom	0	0	0.039598	0	0	0.01276
Ester	0.002009	0.025475	0.006185	0.001229	0.002409	0.003682
Alcohol	0.001184	0.014931	0.00717	0.000726	0.001412	0.000767
Aldehyde	0	0	0.008519	0	0	0.005208
O-arom	0	0	0.006789	0	0	0.003154
N-aliph	0.002403	0	7.73×10^{-5}	0.002689	0	0
O-aliph	0.002399	0	0.001148	0.002685	0	0.001031
Ether	0	0	0.001016	0	0	0.000228

showcase the capabilities of the proposed tool. The code and the data required to obtain the results here present are publicly available in the GitHub subfolder "RCSdata" (of course the "gcms_data_analysis" package must be installed). For conciseness, only a small subset of the results is discussed here.

This case differs from the main example/tutorial in the GitHub repository, as the data presented here is complete and accurate. The GitHub example has the task of showing how to run the module and to perform automatic tests on the code to ensure consistency and thus contains a subset of the data presented here that has been manipulated to make it small and manageable.

Bio-oil samples were produced by dichloromethane extraction of hydrochars, which were in turn produced *via* hydrothermal carbonization at 250 °C for holding time of 1 hour using food waste (named A and Ader in the underivatized and derivatized form, respectively) and cellulose (named B) as feedstock with a 15 and 20% biomass to water ratio. The preparation method for hydrochar production is described in ref. 36. The solvent extraction – performed using an accelerated solvent extractor (ASE Dionex 350) – uses 0.5 g hydrochar and ~25 mL of dichloromethane per sample, with an extraction temperature of 100 °C at ~100 bar. While samples A and B are analyzed after simple dilution, Ader is firstly dried under vacuum, mixed with excess BSTFA, heated at 70 °C for 1 hour, vacuum dried a second time to remove BSTFA, and then reconstituted with dichloromethane before the analysis. The sample are analyzed on a Shimadzu GC 2010 Plus Gas Chromatograph-Mass Spectrometer (GC-MS), equipped with a Restek Rtx-5Sil MS 30 m fused silica column and the semi-quantitative data tables for each sample replicate, the "files_info.xlsx" table (with dilution factor, sample mass in the vial, and name of calibration files), a non-derivatized and a derivatized calibration files, and the "classifications_codes_-fractions.xlsx" files are provided in the "RCSdata" folder.

Table 5 shows a part of the updated semi-quantitative data table results for a single sample, A_1. The "area_if_undiluted" is computed considering the sample dilution of 25× reported in the "files_info" table. The "conc_vial_mg_L" column contains the concentration of each compound in the GC vial, calculated by applying the calibration (and a semi-calibration with Tanimoto similarity threshold of 0.4 and molecular weight

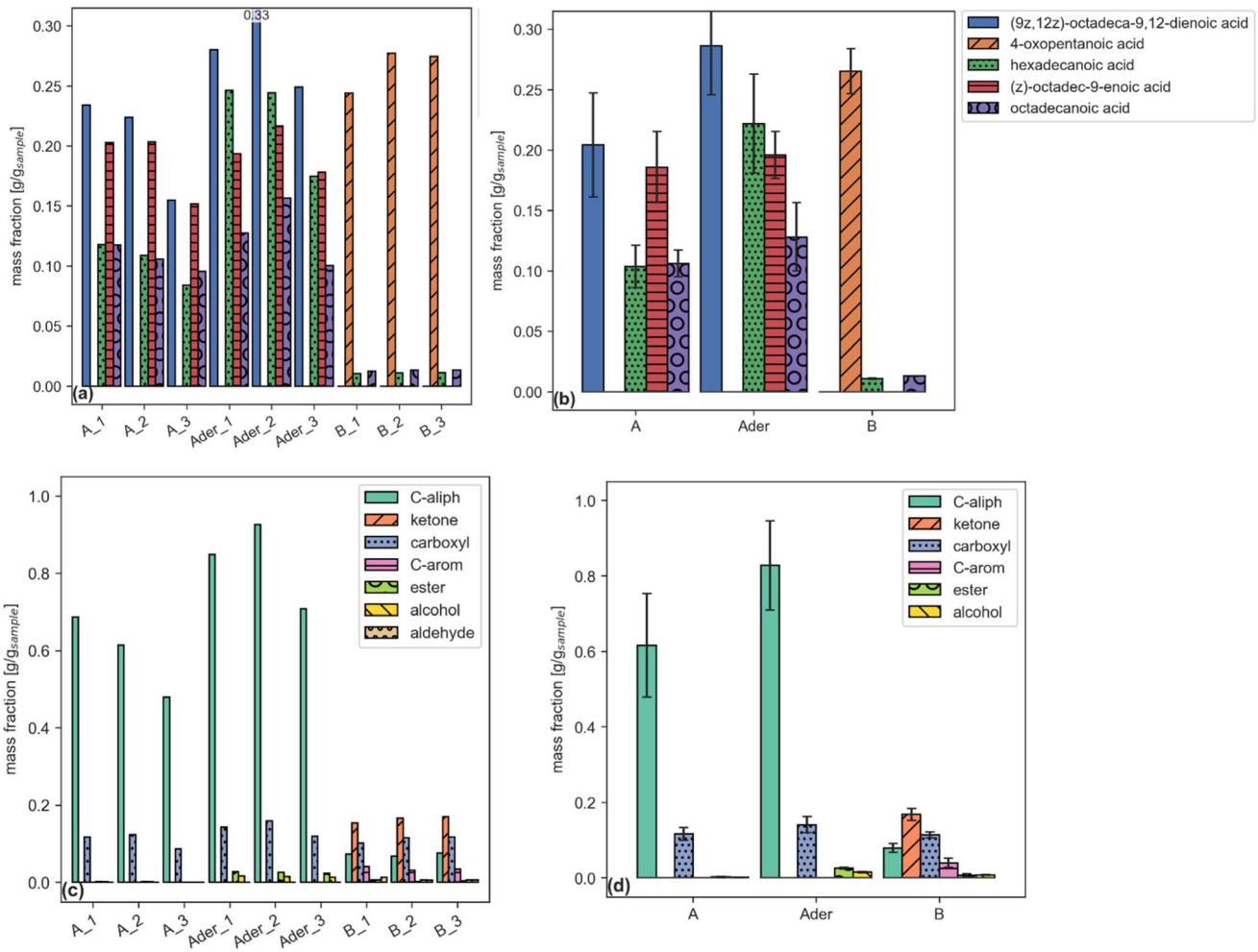


Fig. 2 Single compound (a, b) and functional group aggregated (c, d) plots reporting sample fraction for files (a, c) and samples (b, d). (a and b) share the same legend.

difference threshold of 100). The total concentration of sample in the vial (provided in the "files_info" table) is used to compute the fraction of each compound in the bio-oil sample, shown in the "fraction_of_sample_fr" column in g g^{-1}. The "fraction_of_feedstock_fr" column in g g^{-1} contains the yield of each compound on a feedstock basis, computed using the sample yield vial (provided in the "files_info" table). In this example, the "fraction_of_feedstock_fr" contains the yield of each compound per unit mass of initial biomass.

For each file, an updated report is produced. These results are all collected and summarized into report files for each parameter: Table 6 that shows the first 10 rows of the file report with "conc_vial_mg_L" as the parameter. The algorithm aligns derivatized and non-derivatized instances of the same compound to simplify comparison. Please note that if a non-derivatized and a derivatized calibration are available, the results can be compared, but areas should not be used to describe relative concentrations of a derivatized and underivatized compound. Additional reports with all other parameters can be produced and saved in the "files report" subfolder.

The same reports can be produced using samples instead of files (average and standard deviation of replicates instead of single replicates). In this case, two reports are produced, one with average and one with standard deviation results. Table 7 contains the first 10 rows for average and standard deviation reports for samples using "conc_vial_mg_L" as the parameters (they are directly obtained from Table 5).

The concentrations for each compound in Tables 5 and 6 can be used, together with the mass fraction of each functional group in such compound, to compute aggregated results for each functional group in each sample (using eqn (1), described in Section 2.2.1). Table 8 shows the aggregated results for each functional group in each sample in terms of for sample fraction [g g^{-1}]; additional aggregated reports for the other parameters (area, injection concentration, yield) are also produced by the code but are not reported here.

Each report (an example is given by Tables 6–8) can be plotted by a dedicated function. Examples are shown in Fig. 2.

4 Conclusions

The lack of open-source tools to automate the analysis of large datasets from Gas Chromatography coupled with Mass Spectrometry (GC-MS) of biofuels results in time-consuming manual analyses of such data that employ sub-optimal methodologies, are difficult to replicate, and present an increased risk of error. We developed a Python package to automate GC-MS data analysis of complex, heterogeneous organic mixtures. The code retrieves properties for each identified chemical from PubChem, applies calibration and semi-calibration using Tanimoto similarity index and molecular weight similarity, splits each identified compound into its functional groups, and handles both derivatized and non-derivatized samples. Replicates of the same samples are automatically combined to compute averages and standard deviations. The outputs include single and multiple sample reports with area, concentrations and yield on feedstock basis and aggregated reports based on the cumulative fraction of each functional group in each analyzed sample. This tool reduces GC-MS analysis time from hours days to minutes, avoiding human errors and promoting standardization and best practices for GC-MS data handling. The module is publicly available on PyPI at **https://pypi.org/project/gcms-data-analysis/** and on GitHub at **https://github.com/mpecchi/PyGCMS** with MIT license.

Data availability

The code and the data required to obtain the results here present are publicly available in the GitHub subfolder "RCSdata" at **https://github.com/mpecchi/gcms_data_analysis** (the "gcms_data_analysis" package must be installed **https://pypi.org/project/gcms-data-analysis/**).

Conflicts of interest

There are no conflicts of interest to declare.

Biofuels play a pivotal role in reducing our carbon footprint and transitioning towards a more sustainable energy future. However, the reliance on outdated proprietary software for GC-MS (Gas Chromatography-Mass Spectrometry) analysis not only wastes researchers' time but also hinders comparability across research groups. Our open-source Python tool directly addresses these challenges, ensuring faster, more accurate, and standardized analysis. By democratizing access through open-source software, we also take a stand against knowledge inequality, aligning with the UN's Sustainable Development Goal 10 (Reduced Inequality). Furthermore, our tool contributes to Goal 7 (Affordable and Clean Energy) and Goal 9 (Industry, Innovation, and Infrastructure) as it promotes the adoption of sustainable biofuels and encourages innovation in clean energy research.

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