patRoon: Open source software platform for environmental mass spectrometry based non-target screening

Rick Helmus[a*], Thomas L. ter Laak[a,b], Annemarie P. van Wezel[a], Pim de Voogt[a] and Emma L. Schymanski[c]

[a] Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94240, 1090 GE Amsterdam, The Netherlands

[b] KWR Water Research Institute, Chemical Water Quality and Health, P.O. Box 1072, 3430 BB Nieuwegein, The Netherlands

[c] Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, L-4367 Belvaux, Luxembourg.

* Corresponding author: r.helmus@uva.nl

Abstract

Mass spectrometry based non-target analysis is increasingly adopted in environmental sciences to screen and identify numerous chemicals simultaneously in highly complex samples. However, current data processing software either lack functionality for environmental sciences, solve only part of the workflow, are not openly available and/or are restricted in input data formats. In this paper we present *patRoon*, a new *R* based open-source software platform, which provides comprehensive, fully tailored and straightforward non-target analysis workflows. This platform makes the usage, evaluation and mixing of well-tested algorithms seamless by harmonizing various commonly (primarily open)

software tools under a consistent interface. In addition, *patRoon* offers various functionality and strategies to simplify and perform automated processing of complex (environmental) data effectively. *patRoon* implements several effective optimization strategies to significantly reduce computational times. The ability of *patRoon* to perform a straightforward and effective non-target analysis was demonstrated with real-world environmental samples, showing that *patRoon* makes comprehensive (environmental) non-target analysis readily accessible to a wider community of researchers.

Keywords

High resolution mass spectrometry, compound identification, non-target analysis, computational workflows

Introduction

Chemical analysis is widely applied in environmental sciences such as earth sciences, biology, ecology and environmental chemistry, to study e.g. geomorphic processes, (chemical) interaction between species or the occurrence, fate and effect of chemicals of emerging concern in the environment. The environmental compartments investigated include air, water, soil, sediment and biota, and exhibit a highly diverse chemical composition and complexity. The number and quantities of chemicals encountered within samples may span several orders of magnitude relative to each other. Therefore, chemical analysis must discern compounds at ultra-trace levels, a requirement that can be largely met with modern analytical instrumentation such as liquid or gas chromatography coupled with mass spectrometry (LC-MS and GC-MS). The high sensitivity and selectivity of these

techniques enable accurate identification and quantification of chemicals in complex sample materials.

Traditionally, a 'target analysis' approach is performed, where identification and quantitation occur by comparing experimental data with reference standards. The need to pre-select compounds of interest constrains the chemical scope of target analysis, and hampers the analysis of chemicals with (partially) unknown identities such as transformation products and contaminants of emerging concern (CEC). In addition, the need to acquire or synthesize a large number of analytical standards may not be feasible for compounds with a merely suspected presence. Recent technological advancements in chromatography and high resolution MS (HRMS) allows detection and tentative identification of compounds without the prior need of standards [1]. This 'non-target' analysis (NTA) approach is increasingly adopted to perform simultaneous screening of up to thousands of chemicals in the environment, such as finding new CEC [1–6], identifying chemical transformation (by)products [7–12] and identification of toxicants in the environment [13–16].

Studies employing environmental NTA typically allow the detection of hundreds to thousands of different chemicals [17, 18]. Effectively processing such data requires workflows to automatically extract and prioritize NTA data, perform chemical identification and assist in interpreting the complex resulting datasets. Currently available tools often originate from other research domains such as life sciences and may lack functionality or require extensive optimization before being suitable for environmental analysis. Examples include handling chemicals with low sample-to-sample abundance, recognition of

66 halogenated compounds, usage of data sources with environmentally relevant substances,
67 or temporal and spatial trends. Furthermore, existing tools solve only part of the workflow,
68 generally use differing and incompatible data formats and employ different user interfaces.
69 Hence, the need to learn, combine, optimize and sometimes develop or adapt various
70 specialized software tools, and perform tedious transformation of datasets currently hinders
71 further adoption of NTA, especially in more routine settings lacking appropriate in-house
72 computational expertise.
73
74 An NTA workflow can be generalized as a four step process (Figure 1) [1]. Firstly, data from
75 LC or GC-HRMS is either acquired or retrieved retrospectively, and pre-treated for
76 subsequent analysis (Figure 1a). This pre-treatment may involve conversion to open data
77 formats (e.g. mzML [19] or mzXML [20]) to increase operability with open-source software,
78 re-calibration of mass spectra to improve accuracy and centroiding [21] or other raw data
79 reduction steps to conserve space such as trimming chromatographs or filtering mass scans
80 (e.g. with the functionality from the ProteoWizard suite [22]). Secondly (Figure 1b), features
81 with unique chromatographic and mass spectral properties (e.g. retention time, accurate
82 mass, signal intensity) are automatically extracted and features considered equivalent
83 across sample analyses are grouped to allow qualitative and (semi-) quantitative comparison
84 further down the workflow. Thirdly (Figure 1c), the feature dataset quality is refined, for
85 instance, via rule-based filters (e.g. minimum intensity and absence in sample blanks) and
86 grouping of features based on a defined relationship such as adducts or homologous series
87 (e.g. “componentization”). Further prioritization during this step of the workflow is often
88 required for efficient data analysis, for instance, based on chemical properties (e.g. mass

89 defect and isotopic pattern), suspected presence (i.e. “suspect screening”) or intensity
90 trends in time and/or space (e.g. reviewed in [1]). Finally (Figure 1d), prioritized features are
91 annotated, for instance by assigning chemical formulae or compounds from a chemical
92 database (e.g. *PubChem* [23] or *CompTox* [24]) based on the exact mass of the feature. The
93 resulting candidates are ranked by conformity with MS data, such as match with theoretical
94 isotopic pattern and *in silico* or library MS fragmentation spectra, and study-specific
95 metadata, such as number of scientific references and toxicity data [1, 25].
96
97 Various open and closed software tools are already available to implement (parts of) the
98 NTA workflow. Commercial software tools such as *MetaboScape* [26], *UNIFI* [27], *Compound*
99 *Discoverer* [28] and *ProGenesis QI* [29] provide a familiar and easy to use graphical user
100 interface, may contain instrument specific functionalities and optimizations and typically
101 come with support for their installation and usage. However, they are generally not open-
102 source or open-access and are often restricted to proprietary data formats. This leads to
103 difficulties in data sharing, as exact algorithm implementations and parameter choices are
104 hidden, while maintenance, auditing or code extension by other parties is often not
105 possible. Many open-source or open-access tools are available to process mass
106 spectrometry data (e.g. [30, 31] and summarized in Table 1). While many tools were
107 originally developed to process metabolomics and proteomics data, approaches such as
108 *XCMS* [32] and *MZmine* [33] have also been applied to environmental NTA studies [6, 34].
109 Many open tools are easily interfaced with the *R* statistical environment [35] (Table 1).
110 Leveraging this open scripting environment inherently allows defining highly flexible and
111 reproducible workflows and increases the accessibility of such workflows to a wider

audience as a result of the widespread usage of *R* in data sciences. Various open tools overlap in functionality (Table 1), and are implemented with differing algorithms or employing different data sources. As a consequence, tools may generate different results, as has been shown when generating feature data [36–40] or performing structural annotations [25, 41]. Thus, a flexible platform to combine and evaluate various algorithms that is independent of closed MS vendor input data formats is desired in order to tailor an optimal NTA workflow to the particular study types and methodological characteristics.

Table 1. Overview of commonly used open-source or open-access software tools to implement NTA workflows.

<Table from end of this document should be placed here>

Here, we present an *R* based open-source software platform called *patRoon* ('pattern' in Dutch) providing comprehensive NTA data processing from HRMS data pre-treatment, detection and grouping of features, through to molecular formula and compound annotation. In *patRoon*, various (primarily open) tools commonly used for NTA data processing are harmonized within a consistent and easy to use interface. In addition, new functionality is implemented that simplify and improve NTA data processing, such as automated chemical annotation, visualization and reporting of results, comparing and combining results from different algorithms, and data reduction and prioritization strategies. The architecture of *patRoon* is designed to be extendible in order to accommodate for rapid developments in the NTA research field.

Implementation

The implementation section starts with an overview of the *patRoon* workflows. Subsequent sections provide details on additional functionality implemented by *patRoon* which relate to data processing, annotation, visualization and reporting. Finally, a detailed description is given of the software architecture. *patRoon* is then demonstrated in the Results and discussion section. The software tools and databases used for the implementation of *patRoon* are summarized in Additional file 1.

Workflow in patRoon

patRoon encompasses a comprehensive workflow for HRMS based NTA (Figure 2). All steps within the workflow are optional and the order of execution is largely customizable. Some steps depend on data from previous steps (blue arrows) or may alter or amend data from each other (red arrows). The workflow commonly starts with pre-treatment (PT) of raw HRMS data. Next, feature data is generated, which consists of finding features (FTS) in each sample, an optional retention time alignment step, and then grouping into “feature groups” (FG). FTS and FG may be preceded by automatic parameter optimization (PO), or followed by suspect screening (SUS). The feature data may then finally be used for componentization (CMT) and/or annotation steps, which involves generation of MS peak lists (MSPL), as well as formula and compound annotations (FOR/COM). At any moment during the workflow, the generated data may be inspected, visualized and treated by e.g. rule based filtering. These operations are discussed in the next section.

Several commonly used open software tools, such as *OpenMS* [52], *XCMS* [32], *MetFrag* [48] and *SIRIUS* [54–58], and closed software tools, such as *Bruker DataAnalysis* [61] (chosen due to institutional needs), are interfaced to provide a choice between multiple algorithms for each workflow step (Additional file 3: Table S1). Customization of the NTA workflow may be achieved by freely selecting and mixing algorithms from different software tools. For instance, a workflow that uses *XCMS* to group features allows that these features originate from other algorithms than those supported by *XCMS* (e.g. those from *OpenMS*), a situation that would require tedious data transformation when *XCMS* is used standalone.

To ease parameter selection over the various feature finding and grouping algorithms, an automated feature optimization (FO) approach was adopted from the isotopologue parameter optimization (*IPO*) *R* package [62], which employs design of experiments to optimize LC-MS data processing parameters [63]. IPO was integrated in *patRoon*, and its code base was extended to (a) apply to other feature finding and grouping algorithms supported by *patRoon* (i.e. *XCMS*, *OpenMS* and *enviPick*), (b) support isotope detection with *OpenMS*, (c) perform optimization of qualitative parameters and (d) provide a consistent output format for easy inspection and visualization of optimization results.

In *patRoon*, componentization (CMT) refers to consolidating different (grouped) features with a prescribed relationship, which is currently either based on (a) highly similar elution profiles (i.e. retention time and peak shape), which are hypothesized to originate from the same chemical compound (based on [53, 59]), (b) participation in the same homologous series (based on [64]) or (c) the (normalized) intensity profiles across samples (based on [4,

5, 65]). Components obtained by approach (a) typically comprise adducts, isotopologues and in-source fragments, and the supported algorithms in *patRoon* annotate these using chemical rules. Approach (b) uses the *nontarget R* package [44] to calculate series from aggregated feature data from replicates. The interpretation of homologous series between replicates is assisted by merging series with overlapping features in cases where this will not yield ambiguities to other series. If merging would cause ambiguities, instead links are created that can then be explored interactively and visualized by a network graph generated using the *igraph* [66] and *visNetwork* [67] *R* packages (see Additional file 2: Figure S1).

During the annotation step, molecular formulae and/or chemical compounds are automatically assigned and ranked for all features or feature groups. The required MS peak list (MSPL) input data are extracted from all MS analysis data files and subsequently pre-processed, for instance, by averaging multiple spectra within the elution profile of the feature and by removing mass peaks below user-defined thresholds. All compound databases and ranking mechanisms supported by the underlying algorithms are supported by *patRoon* and can be fully configured. Afterwards, formula and structural annotation data may be combined to improve candidate ranking and manual interpretation of annotated spectra. More details are outlined in the section “MS peak list retrieval, annotation and candidate ranking”.

Data reduction, comparison and conversion

Various rule-based filters are available for data-cleanup or study specific prioritization of all data obtained through the workflow (see Table 2), and can be inverted to inspect the data

that would be removed (i.e. negation). To process feature data, multiple filters are often applied, however, the order may influence the final result. For instance, when features were first removed from blanks by an intensity filter, a subsequent blank filter will not properly remove these features in actual samples. Similarly, a filter may need a re-run after another to ensure complete data clean-up. To reduce the influence of order upon results, filters for feature data are executed by default as follows:

1. an intensity pre-filter, to ensure good quality feature data for subsequent filters;
2. filters not affected by other filters, such as retention time and *m/z* range;
3. minimum replicate abundance, blank presence and 'regular' minimum intensity;
4. repetition of the replicate abundance filter (only if previous filters affected results);
5. other filters that are possibly influenced by prior steps, such as minimum abundance in feature groups or sample analyses.

Note that the above scheme only applies to those filters requested by the user, and the user can apply another order if desired.

Further data subsetting allows the user to freely select data of interest, for instance, following a (statistical) prioritization approach performed by other tools. Similarly, features that are unique or overlapping in different sample analyses may be isolated, which is a straightforward but common prioritization technique for NTA studies that involve the comparison of different types of samples.

Table 2. Major rule-based filtering functionality implemented in patRoon.

Filter functionality	Features		Annotation			Processing
	FTS	FG	MSPL	FOR	COM	CMT
Intensity threshold	X	X	X			
Feature properties[1]	X	X				
Max intensity deviation across replicates		X				
Minimum intensity above blank		X				
Minimum size or abundance		X				X
Top most abundant/highest scoring			X	X	X	
Minimum scoring				X	X	
Annotation[2]				X	X	X
Organic matter rules[3]				X		

FTS: features; FG: feature groups; MSPL: MS peak lists; FOR: formulae; COM: compounds; CMT: components; (1) Retention time, chromatographic peak width, m/z and mass defect range; (2) e.g. adducts, isotopologues, formula composition, neutral loss; (3) expected formula composition based on [68–71].

Data from feature groups, components or annotations that are generated with different algorithms (or parameters thereof) can be compared to generate a consensus by only retaining data with (a) minimum overlap, (b) uniqueness or (c) by combining all results (only (c) is supported for data from components). Consensus data are useful to remove outliers, for inspection of algorithmic differences or for obtaining the maximum amount of data generated during the workflow. The consensus for formula and compound annotation data are generated by comparison of Hill-sorted formulae and the skeleton layer (first block) of the InChIKey chemical identifiers [72], respectively. For feature groups, where different algorithms may output deviating retention and/or mass properties, such a direct comparison is impossible. Instead, the dimensionality of feature groups is first reduced by averaging all feature data (i.e. retention times, *m/z* values and intensities) for each group. The collapsed groups have a similar data format as 'regular' features, where the compared objects represent the 'sample analyses'. Subjection of this data to a feature grouping algorithm supported by *patRoon* (i.e. from *XCMS* or *OpenMS*) then allows straightforward

and reliable comparison of feature data from different algorithms, which is finally used to generate the consensus.

Hierarchical clustering is utilized for componentization of features with similar intensity profiles or to group chemically similar candidate structures of an annotated feature. The latter "compound clustering" assists the interpretation of features with large numbers of candidate structures (e.g. hundreds to thousands). This method utilizes chemical fingerprinting and chemical similarity methods from the *rcdk* package [73] to cluster similar structures, and subsequent visual inspection of the maximum common substructure then allows assessment of common structural properties among candidates (methodology based on [74]). Cluster assignment for both CMT and COM approaches is performed automatically using the *dynamicTreeCut R* package [75]. However, clusters may be re-assigned manually by the desired amount or tree height.

Several data conversion methods were implemented to allow interoperability with other software tools. All workflow data types are easily converted to commonly used *R* data types (e.g. `data.frame` or `list`), which allows further processing with other *R* packages. Furthermore, feature data may be converted to and from native *XCMS* objects (i.e. `xcmsSet` and `XCMSnExp`) or exported to comma-separated values (CSV) formats compatible with *Bruker ProfileAnalysis* or *TASQ*, or *MZmine*.

MS peak list retrieval, annotation and candidate ranking

Data for MS and MS/MS peak lists for a feature are collected from spectra recorded within the chromatographic peak and averaged to improve mass accuracies and signal to noise ratios. Next, peak lists for each feature group are assigned by averaging the mass and intensity values from peak lists of the features in the group. Mass spectral averaging can be customized via several data clean-up filters and a choice between different mass clustering approaches, which allow a trade-off between computational speed and clustering accuracy. By default, peak lists for MS/MS data are obtained from spectra that originate from precursor masses within a certain tolerance of the feature mass. This tolerance in mass search range is configurable to accommodate the precursor isolation window applied during data acquisition. In addition, the precursor mass filter can be completely disabled to accommodate data processing from data-independent MS/MS experiments, where all precursor ions are fragmented simultaneously.

The formula annotation process is configurable to allow a tradeoff between accuracy and calculation speeds. Candidates are assigned to each feature group, either directly by using group averaged MS peak list data, or by a consensus from formula assignments to each individual feature in the group. While the latter inherently consumes more time, it allows removal of outlier candidates (e.g. false positives due to features with poor spectra). Candidate ranking is improved by inclusion of MS/MS data in formula calculation (optional for *GenForm* [47] and *DataAnalysis*).

Formula calculation with *GenForm* ranks formula candidates on isotopic match (amongst others), where any other mass peaks will penalize scores. Since MS data of "real-world" samples typically includes many other mass peaks (e.g. adducts, co-eluting features, background ions), *patRoon* improves the scoring accuracy by automatic isolation of the feature isotopic clusters prior to *GenForm* execution. A generic isolation algorithm was developed, which makes no assumptions on elemental formula compositions and ion charges, by applying various rules to isolate mass peaks that are likely part of the feature isotopic cluster (see Additional file 2: Figure S2). These rules are configured to accommodate various data and study types by default. Optimization is possible, for instance, to (a) improve studies of natural or anthropogenic compounds by lowering or increasing mass defect tolerances, respectively, (b) constrain cluster size and intensity ranges for low molecular weight compounds or (c) adjust to expected instrumental performance such as mass accuracy. Note that precursor isolation can be performed independently of formula calculation, which may be useful for manual inspection of MS data.

Compound annotation is usually the most time and resource intensive process during the non-target workflow. As such, instead of annotating individual features, compound assignment occurs for the complete feature group. All compound databases supported by the underlying algorithms, such as *PubChem* [23], *ChemSpider* [76] or *CompTox* [24] and other local CSV files, as well as the scoring terms present in these databases, such as *in silico* and spectral library MS/MS match, references in literature and presence in suspect lists, can be utilized with *patRoon*. Default scorings supported by the selected algorithm/database or sets thereof are easily selectable to simplify effective compound ranking. Furthermore,

formula annotation data may be incorporated in compound ranking, where a 'formula score' is calculated for each candidate formula, which is proportional to its ranking in the formula annotation data. Execution of unattended sessions is assisted by automatic restarts after occurrence of timeouts or errors (e.g. due to network connectivity) and automatic logging facilities.

Visualization, reporting and graphical interface

In *patRoon*, visualization functionality is provided for feature and annotation data (e.g. extracted ion chromatograms (EICs) and annotated spectra), to compare workflow data (i.e. by means of Venn, chord and UpSet [77] diagrams, using the *VennDiagram* [78], *circlize* [79] and *UpSetR* [80] *R* packages, respectively) and others such as plotting results from automatic feature optimization experiments and hierarchical clustering data. Reports can be generated in a common CSV text format or in a graphical format via export to a portable document file (PDF) or hypertext markup language (HTML) format. The latter are generated with the *R Markdown* [81, 82] and *flexdashboard* [83] *R* packages, and provide an easy to use interface for interactive sorting, searching and browsing reported data. As plotting and reporting functionalities can be performed at any stage during the workflow, the data that is included in the reports is fully configurable.

While *patRoon* is primarily interfaced through *R*, several graphical user interface tools are provided to assist the (novice) user. Most importantly, *patRoon* provides a *Shiny* [84] based tool that automatically generates a commented template *R* script from user input, such as selection of MS data file input, workflow algorithms and other common workflow

parameters (Figure 3a). Secondly, chromatographic data of features may be inspected either by automatic addition of EICs in a *Bruker DataAnalysis* session or with a *Shiny* based interface (Figure 3b).

Software architecture

patRoon is distributed as an *R* package. Its source code is primarily written in the *R* language, with some support code written in C++ and JavaScript. Both *Microsoft Windows* (hereafter referred to as *Windows*) and *Linux* platforms are supported (support for macOS is envisaged in the future). Several external dependencies are required; notable examples are in Additional file 3: Table S1. *GenForm* is automatically compiled during package installation. For *Windows* platforms, an installation script is provided to install and configure *patRoon* and all of its dependencies automatically. Documentation includes a handbook, tutorial and full reference manual [85–88], which are produced with the *bookdown* [89, 90], *R Markdown* and *roxygen2* [91] *R* packages, respectively. Example data is contained in the *patRoonData R* package [92, 93].

An important design goal was to provide a consistent, generic and easy to use interface that does not require the user to know the implementation and interfacing details of the supported algorithms. Each workflow step is executed by a generator function that takes the desired algorithm and its parameters as input and returns objects from a common set of data formats (see Figure 4). Names for commonly used parameters supported by multiple algorithms are standardized for consistency and defaults are set where reasonable. Furthermore, the format of input data such as retention time units as well as formula and

adduct specifications are harmonized and automatically converted to the format expected by the algorithm. Nearly all parameters from the underlying algorithm can be set by the user, hence, full configurability of the workflow is retained wherever possible. Generic naming schemes are applied to output data, which assist the user in comparing results originating from different algorithms. All exported functions from *patRoon* verify user input with the *checkmate* [94] package, which efficiently performs tests such as correctness of value range and type, and prints descriptive messages if input is incorrect.

A set of generic methods are defined for workflow classes that perform general data inspection, selection, conversion and visualization, irrespective of the algorithm that was used to generate the object (see Table 3). Consequently, the implementation of common function names for multiple output classes allows a predictable and consistent user interface.

Table 3. Common generic methods defined in patRoon to process workflow data.

Generic	**Purpose**
`length()`, `show()`, `algorithm()`, `names()`, `groupNames()`	obtain general object information such as object length and unique identifiers for contained results
`filter()`	rule-based filtering operations
`[`, `[[`, `$` operators	subsetting or extracting data
`as.data.table()`, `as.data.frame()`	conversion to `data.table` or `data.frame` object
`unique()`, `overlap()`	extract unique or overlapping features across replicates
`consensus()`	generates a consensus between different objects of the same class
`plot()`, `plotEIC()`, `plotSpec()`	plot general, chromatographic and annotation data
`plotChord()`, `plotUpSet()`,	comparison of feature data or workflow objects from different algorithms by chord, UpSet and Venn diagrams

```
plotVenn()
```

Several optimization strategies are employed in *patRoon* to reduce computational requirements and times. Firstly, external command line (CLI) tools are executed in parallel to reduce overall execution times for repetitive (e.g. per sample analysis or per feature) calculations. Commands are queued (first in, first out) and their execution is handled with the *processx* package [95]. Secondly, functions employing time intensive algorithms automatically cache their (partial) results in a local *SQLite* database file, which is accessed via the *DBI* [96] and *RSQLite* [97] *R* packages. Thirdly, performance critical code dealing with *OpenMS* data files and loading chromatographic data was written in C++ (interfaced with *Rcpp* [98–100]) to significantly reduce times needed to read or write data. Fourthly, the output files from *OpenMS* tools are loaded in chunks using the *pugixml* software library [101] to ensure a low memory footprint. Finally, reading, writing and processing (large) internal tabular data is performed with the *data.table R* package, which is a generally faster and more memory efficient drop-in replacement to the native tabular data format of *R* (`data.frame`), especially for large datasets [102].

Interfacing with *ProteoWizard* [22], *OpenMS*, *GenForm*, *SIRIUS* and *MetFrag* occurs by wrapper code that automatically executes the CLI tools and perform the data conversions necessary for input and output files. An alternative interface to *MetFrag* is also provided by employing the *metfRag R* package [103], however, in our experience this option is currently significantly slower than the CLI and therefore not used by default. For tools that are not readily controllable from *R* (i.e. *ProfileAnalysis*, *TASQ* and *MZmine*), interfacing occurs via importing or exporting CSV files (only export is supported for *MZmine*). Finally, the

383 *RDCOMClient R* package [104] is used to interface with *Bruker DataAnalysis* via the
384 distributed component object model, which allows automation of *DataAnalysis* functionality
385 from *R* that otherwise would only be available via its integrated visual basic scripting
386 environment.
387
388 A continuous integration pipeline performs automated tests during development and
389 delivers files to simplify installation of *patRoon* and all its dependencies (Additional file 2:
390 Figure S3). More than 900 unit tests are performed (>80% code coverage) with the *testthat*
391 and *vdiffr R* packages [105, 106]. After successful test completion, binary *R* packages
392 (*Windows*) and *Docker* images (*Linux*) are generated to facilitate installation of *patRoon* with
393 tested and compatible dependencies.

394 Results and discussion

395 *Benchmark and demonstration data*

396 The data used to benchmark and demonstrate *patRoon* were obtained with an LC-HRMS
397 analysis of two different influent and effluent samples from a drinking water treatment pilot
398 installation and a procedural blank (all in triplicate). The samples originate from an
399 experiment where a set of 18 common environmental contaminants (yielding 20 individual
400 chromatographic peaks, see Additional file 3: Table S2) were spiked prior to drinking water
401 treatment. The analyses were performed using an LC-HRMS Orbitrap Fusion system
402 (ThermoFisher Scientific, Bremen, Germany) operating with positive electrospray ionization.
403 Further analytical conditions are as described in [11]. The raw data files can be obtained
404 from [107].

Parallelization benchmarks

Several benchmarks were performed to test the multiprocessing functionality of *patRoon*. Tests were performed on a personal computer equipped with an Intel® Core™ i7-8700K CPU (6 cores, 12 threads), 32 gigabyte RAM, SATA SSD storage and the *Windows* 10 Enterprise operating system. Benchmarks were performed in triplicate using the *microbenchmark R* package [108]. Standard deviations were below ten percent (see Figure 5a). Benchmarking was performed on *msConvert* (MC), *FeatureFinderMetabo* (FFM), *GenForm* (GF), *SIRIUS* (SIR) and *MetFrag* (MF). The multiprocessing functionality was compared to native multithreading for the tools that supported this (FFM, SIR and MF). In addition, the performance of batch calculations with multiprocessing was compared with native batch calculation modes of tools where possible (MC and SIR). Parallelization methods were tested with 1-12 parallel processes or threads (i.e. up to full utilization of both CPU threads of each core). Input conditions were chosen to simulate "simple" and "complex" workflows, where the latter resulted in more demanding calculations with ~2-10x longer mean execution times (Table 4). The caching functionality of *patRoon* was disabled, where appropriate, to obtain representative and reproducible test results. Prior to benchmarking, candidate chemical compounds from PubChem for MF tests were cached in a local database to exclude influences from network connectivity. Similarly, general spectral data required to post-process FFM results were cached, as this is usually loaded once during a workflow, even with varying input parameters. The input features for GF tests that resulted in very long individual run times (i.e. >30 seconds) were removed to avoid excessive benchmark runtimes. Generating feature and MS peaklist input data for annotation related tests was performed with *patRoon* using algorithms from *OpenMS* and *mzR* [109], respectively. Pre-

treatment of feature data consisted of removal of features with low intensity and lacking MS/MS data. The number of features for SIR (except tests with native batch mode) and MF benchmarks were further reduced by application of blank, replicate and intensity filters to avoid long total runtimes due to their relatively high individual run times. Finally, the feature dataset was split in low (0-500) and high (500-1000) *m/z* portions, which were purposed for execution of "simple" and "complex" experiments, respectively. For more details of the workflow and input parameters see the *R* script code in Additional file 4. The software tools used for benchmarking are summarized in Additional file 1.

Table 4. Utilized conditions for "simple" (S) and "complex" (C) tests.

	Test	Input conditions[1]	Executions	Mean individual run time[2] (s)
msConvert (MC)	MC-S	Conversion centroided input	15	4.8
	MC-C	Centroiding and conversion non-centroided input	15	8.5
FeatureFinderMetabo (FFM)[3]	FFM-S	High intensity threshold	15	4.1
	FFM-C	Low intensity threshold	15	38
GenForm (GF)	GF-S	CHNO elements, low *m/z*	512	0.2
	GF-C	CHNOPS elements, high *m/z*	128	1.7
SIRIUS (SIR)[3]	SIR-S	CHNO elements, low *m/z*	152 (512[4])	2.3
	SIR-C	CHNOPS elements, high *m/z*	44 (128[4])	7.7
MetFrag (MF)[3]	MF-S	Limited scoring, narrow mass search (5 ppm), low *m/z*.	152	3.0
	MF-C	Thorough scoring, wide mass search (20 ppm), high *m/z*.	44	8.6

(1): Features with *m/z* 0 – 500 (low) and *m/z* 500 – 1000 (high); (2): based on a test run without parallelization (n=3); (3) supports (configurable) native multithreading; (4) number of executions for native batch mode benchmarks.

When multiprocessing was used all tests (except GF-S, discussed below) showed a clear downward trend in execution times (down to ~200%-500%), and optimum conditions were

generally reached when the number of parallel processes equaled the number of physical cores (six, see Figure 5a). When algorithms are fully parallelized, execution times are expected to follow an inverse relationship with the number of parallel process (i.e. 1/n) and this was observed most closely with MC, whereas execution times for other tools show a less steep reduction. Furthermore, utilizing multiple threads per core (i.e. hyperthreading) did not reduce execution times further and even slowed down in some cases (e.g. MF-C). These deviations in scalability were not investigated in detail. Since they were more noticeable under complex conditions, it is expected that this may be caused by (a) more involved post-processing results after each execution, which is currently not parallelized, and (b) increased memory usage, which may raise the overhead of context switches performed by the operating system. Nevertheless, the experiments performed here clearly show that the multiprocessing functionality of *patRoon* can significantly reduce execution times of various steps in an NTA workflow.

An exception, however, was the test performed with *GenForm* with simple conditions (GF-S), which exhibited no significant change in execution times with multiprocessing (Figure 5a). Due to the particularly small mean run times (0.2 seconds) of this test, it was hypothesized that the overhead of instantiating a new process from *R* (inherently not parallelized) dominated the overall run times. To mitigate this, a 'batch mode' was implemented, where such process initiation occurs from a command shell sub-process instead. Here, multiple commands are executed by the sub-process in series, and the desired degree of parallelization is then achieved by launching several of these sub-processes and evenly dividing commands amongst them. The maximum size of each series

(or “batch size”) is configurable, and represents a balance between reduction of process initiation overhead and potential loss of effectively load balancing of, for instance, commands with highly deviating execution times. Next, various batch sizes were tested for GF, both with and without multiprocessing parallelization (Additional file 2: Figure S4). For GF-S, execution times clearly decreased with increasing batch sizes, however, no further reduction was observed with parallelism. In contrast, serial execution of GF-C was not affected by varying batch size, whereas added parallelism reduced execution times for small batch sizes (≤8), but significantly increased such times for larger sizes. The results demonstrate that the typical short lived GF executions clearly benefit from batch mode. In addition, it is expected that by further increasing the batch size for GF-S, overall lifetimes of batch sub-processes may increase sufficiently to allow better utilization of parallelization. However, since GF-C results for larger batch sizes clearly show possible performance degradation for more complex calculations (e.g. due to suboptimal load balancing), eight was considered as a ‘safe’ default which improves overall performance for both simple and complex calculation scenarios (Figure 5b).

Utilizing native multithreading for FFM, SIR (without native batch mode) and MF yields only relatively small reductions in their execution times (Figure 5b). Under optimum conditions (6-8 threads), the most significant drop was observed for SIR-C (~40%), followed by FFM-S, FFM-C and MF-C (~20%). These results suggest that native multithreading only yields partial parallelization, which primarily occurs with complex input conditions. Note that *SIRIUS* supports different linear programming solvers (*Gurobi* [110], *CPLEX* [111] and the default *GLPK* [112]), which may influence overall performance and parallelization [113].

Nevertheless, a comparison between these solvers did not reveal significant changes with our experimental conditions (Additional file 2: Figure S5). Combining the multiprocessing functionality with native multithreading under optimum conditions (i.e. 6 parallel processes/threads) only reduces execution times for SIR-C (Figure 5b). As such, both performance improvements and scalability of the multiprocessing implementation of *patRoon* appear highly effective at this stage.

The native batch modes of MC and SIR allow calculations from multiple inputs within a single execution. This reduces the total number of tool executions, which may (1) lower the accumulated overhead associated with starting and finishing tool executions and (2) hamper effective parallelization from multiprocessing, especially if executions are less than the available CPU cores. The combination of multiprocessing (optimum conditions) and native batch mode was benchmarked with increasing number of inputs per tool execution (i.e. the native batch size; Additional file 2: Figure S6). For MC, execution times were largely unaffected by the input batch size if multiprocessing was disabled, which indicates a low execution overhead. Lowest execution times were observed when multiprocessing was enabled with small batch sizes (≤25% of the total inputs), which indicates a lack of native parallelization support. In contrast, SIR showed significantly lower overall execution times with increasing batch sizes (up to ~7000% and ~320% for SIR-S and SIR-C, respectively), while enabling multiprocessing did not reduce execution times for batch sizes >1. These results show that (1) SIR has a relative large execution overhead, which impairs multiprocessing performance gains, and (2) supports effective native parallelized batch execution. Thus, SIR performs most optimal if all calculations are performed within a single

execution. Similar to previous SIR benchmarks, no significant differences were found across different linear solvers (Additional file 2: Figure S7). The results demonstrate that multiprocessing may improve efficiency for batch calculations with tools with low execution overhead and/or lack of native parallelization. Nonetheless, the dramatic improvement in SIR calculation times when using the native batch mode indicates that software authors should generally consider implementing native threaded batch mode functionality if large batch calculations are an expected use case.

Finally, the implemented optimization strategies were tested for a complete *patRoon* NTA workflow consisting of typical data processing steps and using all previously tested tools. The chosen input conditions roughly fell in between the aforementioned “simple” and “complex” conditions (see code in Additional file 4). Note that optimization strategies were unavailable for some steps (e.g. grouping of features and collection of MS peak lists), and native batch mode was not used in order to demonstrate the usefulness of multiprocessing for tools that do not support this (e.g. other tools than MC and SIR and those potentially available in future versions of *patRoon*). Regardless, the benchmarks revealed a reduction in total run times of ~50% (from ~200 to ~100 minutes; Figure 5c). Since execution times of each step may vary significantly, the inclusion of different combinations of steps may significantly influence overall execution times.

The use of multiprocessing for all tools (except SIR), the implemented batch mode strategies for GF and the use of the native batch mode supported by SIR were set as default in *patRoon* with the determined optimal parameters from the benchmarks results. However,

the user can still freely configure all these options to potentially apply further optimizations or otherwise (partially) disable parallelization to conserve system resources acquired by *patRoon*.

As a final note, it is important to realize that these benchmarks display execution times that also involve preparing and processing results and include other overhead such as process creation from *R*. For this reason, a direct comparison with standalone execution of investigated tools was not possible. Nevertheless, the various optimization strategies employed by *patRoon* minimize such overhead, and the added parallelization functionality often provide a clear advantage in efficiency when using typical CLI tools in an *R* based NTA workflow, especially considering the now widespread availability of computing systems with increasing numbers of cores.

Demonstration: suspect screening

The previous section investigated several parallelization strategies implemented in *patRoon* for efficient data processing. A common method in environmental NTA studies to increase data processing efficiency and reducing the data complexity is by merely screening for chemicals of interest. This section demonstrates such a suspect screening workflow with *patRoon*, consisting of (a) raw data pre-treatment, (b) extracting, grouping and suspect screening of feature data, and finally (c) annotating features to confirm their identity. During the workflow several rule-based filters are applied to improve data quality. The 'suspects' in this demonstration are, in fact, a set of compounds spiked to influent samples (Additional file 3: Table S2), hence, they were used for validation purposes of the workflow. After

completion of the suspect screening workflow, several methods are demonstrated to inspect the resulting data.

Suspect screening: workflow

The code described here can easily be generated with the `newProject()` function, which automatically generates a ready-to-use R script based on user input (section "Visualization, reporting and graphical interface").

First, the *patRoon R* package is loaded and a `data.frame` is generated with the file information of the sample analyses and their replicate and blank assignments. Next, this information is used to centroid and convert the raw analyses files to the open mzML file format, a necessary step for further processing.

```
library(patRoon)

# Generate analysis file information for all files in a directory,
# assign replicate group names to all triplicates and specify which
# should be used for blank subtraction.
anaInfo <- generateAnalysisInfo("../data",
                                groups = c(rep("blank", 3),
                                           rep("influent-A", 3),
                                           rep("effluent-A", 3),
                                           rep("influent-B", 3),
                                           rep("effluent-B", 3)),
                                blanks = "blank")

convertMSFiles(anaInfo = anaInfo, from = "thermo", to = "mzML",
               algorithm = "pwiz", centroid = "vendor")
```

The next step involves finding features and grouping them across samples. This example uses the *OpenMS* algorithms and sets several algorithm specific parameters that were manually optimized for the employed analytical instrumentation to optimize the workflow output. Other algorithms (e.g. *enviPick*, *XCMS*) are easily selected by changing the `algorithm` function parameter.

```
features <- findFeatures(anaInfo, algorithm = "openms",
                         noiseThrInt = 4E3,
                         chromFWHM = 3, minFWHM = 1, maxFWHM = 30,
                         chromSNR = 5, mzPPM = 5)
fGroups <- groupFeatures(features, algorithm = "openms")
```

Several rule-based filters are then applied for general data clean-up, followed by the removal of sample blanks from the feature dataset.

```
fGroups <- filter(fGroups,
                  # minimum absolute feature intensity
                  absMinIntensity = 1E5,
                  # must be present in all replicates
                  relMinReplicateAbundance = 1,
                  # max relative standard deviation replicate intensities
                  maxReplicateIntRSD = 0.75,
                  # minimum feature intensity above blank
                  blankThreshold = 5,
                  # remove blank analyses afterwards
                  removeBlanks = TRUE)
```

Next, features are screened with a given suspect list, which is a CSV file read into a `data.frame` containing the name, SMILES and (optionally) retention time for each suspect (see Additional file 5). While the list in this demonstration is rather small (18 compounds, see SX), larger lists containing several thousands of compounds such as those available on the NORMAN network Suspect List Exchange [114] can also be used. The screening results are returned in a `data.frame`, where each row is a hit (a suspect may occur multiple times) containing the linked feature group identifier and other information such as detected *m/z* and retention time (deviations). Finally, this table is used to transform the original feature groups object (`fGroups`) by removing any unassigned features and tagging remainders by their suspect name.

```
suspects <- read.csv("suspects.csv")
scr <- screenSuspects(fGroups, suspects, mzWindow = 0.002,
                      rtWindow = 6, adduct = "[M+H]+")
fGroupsSusp <- groupFeaturesScreening(fGroups, scr)
```

In the final step of this workflow annotation is performed, which consists of (a) generation of MS peak list data, (b) general clean-up to only retain significant MS/MS mass peaks, automatic annotation of (c) formulae and (d) chemical compounds, and (e) combining both annotation data to improve ranking of candidate compounds. As with previous workflow steps, the desired algorithms (*mzR*, *GenForm* and *MetFrag* in this example) are set using the `algorithm` function parameter. Similarly, the compound database used by *MetFrag* (here *CompTox* via a local CSV file obtained from [115]) can easily be changed to other databases such as *PubChem*, *ChemSpider* or another local file.

```
mslists <- generateMSPeakLists(fGroupsSusp, "mzr",
                               precursorMzWindow = 0.5)
mslists <- filter(mslists, relMSMSIntThr = 0.02, topMSMSPeaks = 10)

formulas <- generateFormulas(fGroupsSusp, "genform", mslists,
                             adduct = "[M+H]+",
                             elements = "CHNOPSClBr")
# Configure location of CompTox CSV file
options(patRoon.path.MetFragCompTox =
            "C:/CompTox_17March2019_SelectMetaData.csv")
compounds <- generateCompounds(fGroupsSusp, mslists, "metfrag",
                               adduct = "[M+H]+",
                               database = "comptox")
compounds <- addFormulaScoring(compounds, formulas, updateScore = TRUE)
```

Suspect screening: data inspection

All data generated during the workflow (e.g. features, peak lists, annotations) can be inspected by overloads of common *R* methods.

```
# intensities for each feature in first group
> fGroups[[1]]
[1]  210235.3  242051.9  254323.8  260419.1  205407.0  261099.1         0.0
0.0       0.0       0.0       0.0       0.0

# averaged MS/MS peak list for feature group of carbamazepine suspect
> mslists[["Carbamazepine"]]$MSMS
mz           intensity    precursor
1: 192.0804  284478.607     FALSE
2: 193.0880   69396.510     FALSE
3: 194.0960 1126534.943     FALSE
4: 237.1019    5406.667      TRUE

# compound annotation data for all features (subset shown for clarity)
> as.data.frame(compounds)[1:5, 1:5]
group              explainedPeaks score neutralMass  SMILES
1 n-Methylbenzotriazole-1  4 12.268046  133.064   NC1=NC2=CC=CC=C2N1
2 n-Methylbenzotriazole-1  5  9.546212  133.064 CC1=CC2=C(NN=N2)C=C1
3 n-Methylbenzotriazole-1  5  6.722034  133.064   NC1=CC=C2NN=CC2=C1
4 n-Methylbenzotriazole-1  5  6.715495  133.064   CC1=C2NN=NC2=CC=C1
5 n-Methylbenzotriazole-1  4  6.483770  133.064   CN1N=NC2=CC=CC=C12
```

Furthermore, all workflow data can easily be subset with e.g. the *R* subset operator ("[“), for instance, to perform a (hypothetical) prioritization of features that are most intense in the effluent samples.

```
# obtain table with replicate averaged feature intensities
> intTab <- as.data.frame(fGroupsSusp, average = TRUE)
> head(intTab)[, 1:5] # show first 5 rows/columns
group                      ret       mz       influent-A effluent-A
1 n-Methylbenzotriazole-1 600.6524 134.0709  2021597.7        0.0
2 n-Methylbenzotriazole-2 607.5665 134.0709  2399435.6   192759.6
3                 Barbital 137.3162 185.0918   145150.0        0.0
4           Benzotriazole 478.6665 120.0553  1494092.0   190069.0
5           Carbamazepine 797.5051 237.1018  2849756.3        0.0
6             Carbendazim 378.8226 192.0764   504191.7        0.0

# obtain group names from the 5 highest intense features in either
# of the effluents
> top1 <- intTab$group[order(intTab[["effluent-A"]],
                             decreasing = TRUE)][1:5]
> top2 <- intTab$group[order(intTab[["effluent-B"]],
                             decreasing = TRUE)][1:5]
> top <- union(top1, top2)
> top
[1] "Metformin"                "Terbuthylazine"
[3] "Triphenylphosphine oxide" "Melamine-2"
[5] "n-Methylbenzotriazole-2"  "Benzotriazole"
[7] "n-Methylbenzotriazole-1"  "Propranolol"

# subset original object
> fGroupsSusp <- fGroupsSusp[, top]
```

Visualization of data generated during the workflow is performed by various plotting functions (see Figure 6).

```
# plot unique features in influents
plot(fGroups[rGroups = c("influent-A", "influent-B")],
     colourBy = "rGroups", onlyUnique = TRUE)
# all EICs for a feature group
plotEIC(fGroupsSusp[, "Terbuthylazine"], colourBy = "rGroup")
plotSpec(compounds, index = 1, groupName = "Benzotriazole",
         mslists)
plotUpSet(fGroupsSusp)
plotChord(fGroupsSusp, average = TRUE)
plotVenn(fGroupsSusp, which = c("influent-B", "effluent-B"))
```

The final step in a *patRoon* NTA workflow involves automatic generation of comprehensive reports of various formats which allow (interactive) exploration of all data (see Additional file 2: Figure S8).

```
reportCSV(fGroupsSusp, formulas = formulas, compounds = compounds)
reportPDF(fGroupsSusp, formulas = formulas,
          compounds = compounds, MSPeakLists = mslists)
reportHTML(fGroupsSusp, formulas = formulas,
           compounds = compounds, MSPeakLists = mslists)
```

Suspect screening: results

A summary of data generated during the NTA workflow demonstrated here is shown in Table 5 and Additional file 3: Table S2. The complete workflow finished in approximately 8 minutes (employing a laptop with an Intel® Core™ I7-8550U CPU, 16 gigabyte RAM, NVME SSD and the Windows 10 Pro operating system). While nearly 60 000 features were grouped into nearly 20 000 feature groups, the majority (97%, 678 remaining) were filtered out during the various pre-treatment filter steps. Regardless, most suspects were found (17/18 attributed to 19/20 individual chromatographic peaks), and the missing suspect (aniline) could be detected when lowering the intensity threshold of the `filter()` function used to post-filter feature groups in the workflow. The majority of suspects (17) were annotated with the correct chemical compound as first candidate, the two n-methylbenzotriazole

621 isomer suspects were ranked as second or fourth. Results for formulae assignments were
622 similar, with the exception of dimethomorph, where the formula was ranked in only the top
623 twenty-five (the candidate chemical compound was ranked first, however).
624
625 While this demonstration conveys a relative simple NTA with 'known suspects', the results
626 show that *patRoon* (a) allows a straightforward approach to perform a complete and
627 tailored NTA workflow, (b) provides powerful general data clean-up functionality to
628 prioritize data and (c) realizes effective automated annotation of detected features.
629
630 *Table 5. Summarizing results for the demonstrated patRoon NTA workflow.*

		Amount
Features	Total found	57 113 (mean 3,808/sample)
Feature groups	Raw dataset	19 970
	Replicate filters (1st pass[1])	4 719 (-76%)
	Blank filter	2 933 (-85%)
	Intensity filters	964 (-95%)
	Replicate filters (2nd pass[1])	678 (-97%)
Suspects	Total found	19 out of 20
	Annotated	19
Formulae	Total candidates	163 (mean 9/feature group)
	Correctly ranked 1st	13 (68%)
	Correctly ranked 1st-2nd	16 (84%)
	Correctly ranked 1st-5th	17 (89%)
Compounds	Total candidates	1 017 (mean 54/feature group)
	Correctly ranked 1st	17 (85%)
	Correctly ranked 1st-2nd	18 (90%)
	Correctly ranked 1st-5th	19 (100%)

(1): Replicate filters are repeated if necessary, see section "Data reduction, comparison and conversion".

631 # Conclusions

632 This paper presents *patRoon*, a fully open source platform that provides a comprehensive
633 MS based NTA data processing workflow developed in the *R* environment. Major workflow

634 functionality is implemented through the usage of existing and well-tested software tools,
635 connecting primarily open and a few closed approaches. The workflows are easily setup for
636 common use cases, while full customization and mixing of algorithms allows for execution of
637 completely tailored workflows. In addition, extensive functionality related to data
638 processing, annotation, visualization, reporting and others was implemented in *patRoon* to
639 provide an important toolbox for effectively handling complex NTA studies. The easy and
640 predictable interface of *patRoon* lowers the computational expertise required of users,
641 making it available for a broad audience. Major implemented optimization strategies were
642 demonstrated to reduce computational times. Furthermore, a typical suspect screening
643 workflow was demonstrated on real-world data from an environmental study related to
644 drinking water treatment.
645
646 *patRoon* has been under development for several years and has already been applied in a
647 variety of studies, such as the characterization of organic matter [71], elucidation of
648 transformation products of biocides [7, 12] and assessment of removal of polar organics
649 reversed-osmosis drinking water treatment [14]. *patRoon* will undergo further
650 development, and extension of integrated workflow algorithms is planned for new and less
651 commonly used ones, while additional componentization strategies will be implemented to
652 help prioritizing data. Addition of new workflow functionality is foreseen, such as usage of
653 ion-mobility spectrometry data to assist annotation, automated screening of transformation
654 products (e.g. utilizing tools such as *BioTransformer* [116]), prediction of feature quantities
655 for prioritization purposes (recently reviewed in [117]) and automated chemical
656 classification (e.g. through *ClassyFire* [118]). Finally, interfacing with other *R* based mass

spectrometry software such as those provided by the "R for Mass Spectrometry" initiative [119] is planned to further improve the interoperability of *patRoon*. The use in real-world studies, feedback from users and developments within the non-target analysis community, are all critical in determining future directions and improvements of *patRoon*. We envisage that the open availability, straightforward usage, vendor independence and comprehensive functionality will be useful to the community and result in a broad adoption of *patRoon*.

Availability and requirements

Project name: patRoon

Project home page: https://github.com/rickhelmus/patRoon

Operating system(s): Platform independent (tested on Microsoft Windows and Linux)

Programming language(s): R, C++, JavaScript

Other requirements: Depending on utilized algorithms (see installation instructions in [85, 88])

License: GNU GPL version 3

Any restrictions to use by non-academics: none

Abbreviations

CEC: Chemical of emerging concern

CLI: Command-line interface

CMP: Compound annotation

CMT: Componentization

CSV: Comma-separated value

DBI: The database interface

EIC: Extracted ion chromatogram

FFM(-S/C): FeatureFinderMetabo (simple/complex conditions)

FG: Feature groups

FOR: Formula annotation

FTS: Features

GC: Gas chromatography

GC-MS: GC coupled to mass spectrometry

GF(-S/C): GenForm (simple/complex conditions)

HTML: Hypertext markup language

HRMS: High resolution mass spectrometry

IPO: Isotopologue parameter optimization

LC: Liquid chromatography

LC-MS: LC coupled to mass spectrometry

MC(-S/C): msConvert (simple/complex conditions)

MF(-S/C): MetFrag (simple/complex conditions)

MS/MS: Tandem mass spectrometry

MSPL: MS peak list

NTA: Non-target analysis

PDF: Portable document format

PO: Parameter optimization

PT: Pre-treatment

SIR(-S/C): SIRIUS (simple/complex conditions)

SUS: Suspect screening

XCMS: Various forms (X) of chromatography mass spectrometry (*R* package MS data processing)

Definitions

Features (FTS): data points assigned with unique chromatographic and mass spectral information (e.g. retention time, peak area and accurate m/z), which potentially described a compound in a sample analysis.

Feature group (FG): A group of features considered equivalent across sample analyses.

MS peak list (MSPL): tabular data (*m/z* and intensity) for MS or MS/MS peaks attributed to a feature and used as input data for annotation purposes.

Formula/Compound (FOR/CMP): a chemical formula or compound candidate revealed during feature annotation.

Component (CMT): A collection of feature groups that are somehow linked, such as MS adducts, homologous series or highly similar intensity trends.

Declarations

Availability of data and materials

The source code of *patRoon* and online versions of its manuals are available for download from https://github.com/rickhelmus/patRoon and archived in [120]. The raw data used for benchmarking and demonstration purposes in this manuscript is archived in [107]. The scripts used to perform benchmarking and the input suspect list for demonstration purposes are provided as Additional file 4 and 5, respectively.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was internally funded by the Institute of Biodiversity and Ecosystem Dynamics (University of Amsterdam). ELS is supported by the Luxembourg National Research Fund (FNR) for project A18/BM/12341006.

Authors' contributions

RH wrote the manuscript, source code, designed the experiments and interpreted the results. ELS provided valuable feedback to improve the software. ELS and other authors supervised this work and contributed to writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The many authors involved in the open mass spectrometry software development community are highly acknowledged as their contributions are the foundation for the development of *patRoon*. In addition, Vittorio Albergamo, Andrea Brunner, Thomas Wagner, Olaf Brock and other users of *patRoon* are thanked for testing and providing feedback for future developments. We thank the Dutch drinking water companies Dunea and PWN for sharing the raw HRMS data that was used for benchmarking and demonstration purposes. Markus Fleischauer is acknowledged for his feedback on execution of batch execution of *SIRIUS*. Finally, Olaf Brock is acknowledged for the design of some of the visualizations of benchmarking data.

References

1. Hollender J, Schymanski EL, Singer HP, Ferguson PL (2017) Nontarget Screening with High Resolution Mass Spectrometry in the Environment: Ready to Go? Environ Sci Technol 51:11505–11512 . https://doi.org/10.1021/acs.est.7b02184
2. Chiaia-Hernandez AC, Schymanski EL, Kumar P, Singer HP, Hollender J (2014) Suspect and nontarget screening approaches to identify organic contaminant records in lake sediments. Anal Bioanal Chem 406:7323–7335 . https://doi.org/10.1007/s00216-014-8166-0
3. Sjerps RMA, Vughs D, van Leerdam JA, ter Laak TL, van Wezel AP (2016) Data-driven prioritization of chemicals for various water types using suspect screening LC-HRMS. Water Research 93:254–264 . https://doi.org/10.1016/j.watres.2016.02.034
4. Chiaia-Hernández AC, Günthardt BF, Frey MP, Hollender J (2017) Unravelling Contaminants in the Anthropocene Using Statistical Analysis of Liquid Chromatography–High-Resolution Mass Spectrometry Nontarget Screening Data Recorded in Lake Sediments. Environ Sci Technol 51:12547–12556 . https://doi.org/10.1021/acs.est.7b03357
5. Albergamo V, Schollée JE, Schymanski EL, Helmus R, Timmer H, Hollender J, de Voogt P (2019) Nontarget Screening Reveals Time Trends of Polar Micropollutants in a Riverbank Filtration System. Environ Sci Technol 53:7584–7594 . https://doi.org/10.1021/acs.est.9b01750
6. Hernández F, Bakker J, Bijlsma L, de Boer J, Botero-Coy AM, Bruinen de Bruin Y, Fischer S, Hollender J, Kasprzyk-Hordern B, Lamoree M, López FJ, Laak TL ter, van Leerdam JA, Sancho JV, Schymanski EL, de Voogt P, Hogendoorn EA (2019) The role of analytical

766 chemistry in exposure science: Focus on the aquatic environment. Chemosphere
767 222:564–583 . https://doi.org/10.1016/j.chemosphere.2019.01.118
768 7. Wagner TV, Helmus R, Quiton Tapia S, Rijnaarts HHM, de Voogt P, Langenhoff AAM,
769 Parsons JR (2020) Non-target screening reveals the mechanisms responsible for the
770 antagonistic inhibiting effect of the biocides DBNPA and glutaraldehyde on benzoic
771 acid biodegradation. Journal of Hazardous Materials 386:121661 .
772 https://doi.org/10.1016/j.jhazmat.2019.121661
773 8. Kolkman A, Martijn BJ, Vughs D, Baken KA, van Wezel AP (2015) Tracing Nitrogenous
774 Disinfection Byproducts after Medium Pressure UV Water Treatment by Stable Isotope
775 Labeling and High Resolution Mass Spectrometry. Environ Sci Technol 49:4458–4465 .
776 https://doi.org/10.1021/es506063h
777 9. Schollée JE, Schymanski EL, Avak SE, Loos M, Hollender J (2015) Prioritizing Unknown
778 Transformation Products from Biologically-Treated Wastewater Using High-Resolution
779 Mass Spectrometry, Multivariate Statistics, and Metabolic Logic. Anal Chem 87:12121–
780 12129 . https://doi.org/10.1021/acs.analchem.5b02905
781 10. Brunner AM, Vughs D, Siegers W, Bertelkamp C, Hofman-Caris R, Kolkman A, ter Laak T
782 (2019) Monitoring transformation product formation in the drinking water treatments
783 rapid sand filtration and ozonation. Chemosphere 214:801–811 .
784 https://doi.org/10.1016/j.chemosphere.2018.09.140
785 11. Brunner AM, Bertelkamp C, Dingemans MML, Kolkman A, Wols B, Harmsen D, Siegers
786 W, Martijn BJ, Oorthuizen WA, ter Laak TL (2020) Integration of target analyses, non-
787 target screening and effect-based monitoring to assess OMP related water quality

changes in drinking water treatment. Science of The Total Environment 705:135779 . https://doi.org/10.1016/j.scitotenv.2019.135779

12. Wagner TV, Helmus R, Becker E, Rijnaarts HHM, Voogt P de, Langenhoff AAM, Parsons JR (2020) Impact of transformation, photodegradation and interaction with glutaraldehyde on the acute toxicity of the biocide DBNPA in cooling tower water. Environ Sci: Water Res Technol 6:1058–1068 . https://doi.org/10.1039/C9EW01018A

13. Jonker W, Lamoree MH, Houtman CJ, Hamers T, Somsen GW, Kool J (2015) Rapid activity-directed screening of estrogens by parallel coupling of liquid chromatography with a functional gene reporter assay and mass spectrometry. Journal of Chromatography A 1406:165–174 . https://doi.org/10.1016/j.chroma.2015.06.012

14. Albergamo V, Escher BI, Schymanski EL, Helmus R, Dingemans MML, Cornelissen ER, Kraak MHS, Hollender J, Voogt P de (2019) Evaluation of reverse osmosis drinking water treatment of riverbank filtrate using bioanalytical tools and non-target screening. Environ Sci: Water Res Technol 6:103–116 . https://doi.org/10.1039/C9EW00741E

15. Brunner AM, Dingemans MML, Baken KA, van Wezel AP (2019) Prioritizing anthropogenic chemicals in drinking water and sources through combined use of mass spectrometry and ToxCast toxicity data. Journal of Hazardous Materials 364:332–338 . https://doi.org/10.1016/j.jhazmat.2018.10.044

16. Zwart N, Jonker W, Broek R ten, de Boer J, Somsen G, Kool J, Hamers T, Houtman CJ, Lamoree MH (2020) Identification of mutagenic and endocrine disrupting compounds in surface water and wastewater treatment plant effluents using high-resolution

effect-directed analysis. Water Research 168:115204 . https://doi.org/10.1016/j.watres.2019.115204

17. Schymanski EL, Singer HP, Slobodnik J, Ipolyi IM, Oswald P, Krauss M, Schulze T, Haglund P, Letzel T, Grosse S, Thomaidis NS, Bletsou A, Zwiener C, Ibáñez M, Portolés T, de Boer R, Reid MJ, Onghena M, Kunkel U, Schulz W, Guillon A, Noyon N, Leroy G, Bados P, Bogialli S, Stipaničev D, Rostkowski P, Hollender J (2015) Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis. Anal Bioanal Chem 407:6237–6255 . https://doi.org/10.1007/s00216-015-8681-7

18. Peisl BYL, Schymanski EL, Wilmes P (2018) Dark matter in host-microbiome metabolomics: Tackling the unknowns–A review. Analytica Chimica Acta 1037:13–27 . https://doi.org/10.1016/j.aca.2017.12.034

19. Martens L, Chambers M, Sturm M, Kessner D, Levander F, Shofstahl J, Tang WH, Römpp A, Neumann S, Pizarro AD, Montecchi-Palazzi L, Tasman N, Coleman M, Reisinger F, Souda P, Hermjakob H, Binz P-A, Deutsch EW (2011) mzML—a Community Standard for Mass Spectrometry Data. Molecular & Cellular Proteomics 10: . https://doi.org/10.1074/mcp.R110.000133

20. Pedrioli PGA, Eng JK, Hubley R, Vogelzang M, Deutsch EW, Raught B, Pratt B, Nilsson E, Angeletti RH, Apweiler R, Cheung K, Costello CE, Hermjakob H, Huang S, Julian RK, Kapp E, McComb ME, Oliver SG, Omenn G, Paton NW, Simpson R, Smith R, Taylor CF, Zhu W, Aebersold R (2004) A common open representation of mass spectrometry data and its application to proteomics research. Nat Biotechnol 22:1459–1466 . https://doi.org/10.1038/nbt1031

833 21. Urban J, Afseth NK, Štys D (2014) Fundamental definitions and confusions in mass
834 spectrometry about mass assignment, centroiding and resolution. TrAC Trends in
835 Analytical Chemistry 53:126–136 . https://doi.org/10.1016/j.trac.2013.07.010
836 22. Chambers MC, Maclean B, Burke R, Amodei D, Ruderman DL, Neumann S, Gatto L,
837 Fischer B, Pratt B, Egertson J, Hoff K, Kessner D, Tasman N, Shulman N, Frewen B, Baker
838 TA, Brusniak M-Y, Paulse C, Creasy D, Flashner L, Kani K, Moulding C, Seymour SL,
839 Nuwaysir LM, Lefebvre B, Kuhlmann F, Roark J, Rainer P, Detlev S, Hemenway T,
840 Huhmer A, Langridge J, Connolly B, Chadick T, Holly K, Eckels J, Deutsch EW, Moritz RL,
841 Katz JE, Agus DB, MacCoss M, Tabb DL, Mallick P (2012) A cross-platform toolkit for
842 mass spectrometry and proteomics. Nat Biotechnol 30:918–920 .
843 https://doi.org/10.1038/nbt.2377
844 23. PubChem National Center for Biotechnology Information PubChem Database.
845 https://pubchem.ncbi.nlm.nih.gov/. Accessed 6 Feb 2020
846 24. Williams AJ, Grulke CM, Edwards J, McEachran AD, Mansouri K, Baker NC, Patlewicz G,
847 Shah I, Wambaugh JF, Judson RS, Richard AM (2017) The CompTox Chemistry
848 Dashboard: a community data resource for environmental chemistry. Journal of
849 Cheminformatics 9:61 . https://doi.org/10.1186/s13321-017-0247-6
850 25. Blaženović I, Kind T, Torbašinović H, Obrenović S, Mehta SS, Tsugawa H, Wermuth T,
851 Schauer N, Jahn M, Biedendieck R, Jahn D, Fiehn O (2017) Comprehensive comparison
852 of in silico MS/MS fragmentation tools of the CASMI contest: database boosting is
853 needed to achieve 93% accuracy. Journal of Cheminformatics 9:32 .
854 https://doi.org/10.1186/s13321-017-0219-x

855 26. Bruker MetaboScape. https://www.bruker.com/products/mass-spectrometry-and-
856 separations/ms-software/metaboscape.html. Accessed 6 Feb 2020
857 27. Waters UNIFI Scientific Information System.
858 https://www.waters.com/waters/en_US/UNIFI-Scientific-Information-
859 System/nav.htm?cid=134801359&locale=en_US. Accessed 6 Feb 2020
860 28. Thermo Scientific Compound Discoverer Software.
861 https://www.thermofisher.com/uk/en/home/industrial/mass-spectrometry/liquid-
862 chromatography-mass-spectrometry-lc-ms/lc-ms-software/multi-omics-data-
863 analysis/compound-discoverer-software.html. Accessed 6 Feb 2020
864 29. Progenesis QI. http://www.nonlinear.com/progenesis/qi/. Accessed 6 Feb 2020
865 30. Misra BB, Mohapatra S (2019) Tools and resources for metabolomics research
866 community: A 2017–2018 update. ELECTROPHORESIS 40:227–246 .
867 https://doi.org/10.1002/elps.201800428
868 31. Stanstrup J, Broeckling CD, Helmus R, Hoffmann N, Mathé E, Naake T, Nicolotti L,
869 Peters K, Rainer J, Salek RM, Schulze T, Schymanski EL, Stravs MA, Thévenot EA,
870 Treutler H, Weber RJM, Willighagen E, Witting M, Neumann S (2019) The
871 metaRbolomics Toolbox in Bioconductor and beyond. Metabolites 9:200 .
872 https://doi.org/10.3390/metabo9100200
873 32. Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G (2006) XCMS: Processing Mass
874 Spectrometry Data for Metabolite Profiling Using Nonlinear Peak Alignment, Matching,
875 and Identification. Anal Chem 78:779–787 . https://doi.org/10.1021/ac051437y

876 33. Pluskal T, Castillo S, Villar-Briones A, Orešič M (2010) MZmine 2: Modular framework
877 for processing, visualizing, and analyzing mass spectrometry-based molecular profile
878 data. BMC Bioinformatics 11:395 . https://doi.org/10.1186/1471-2105-11-395
879 34. Hohrenk LL, Itzel F, Baetz N, Tuerk J, Vosough M, Schmidt TC (2020) Comparison of
880 Software Tools for Liquid Chromatography–High-Resolution Mass Spectrometry Data
881 Processing in Nontarget Screening of Environmental Samples. Anal Chem 92:1898–
882 1907 . https://doi.org/10.1021/acs.analchem.9b04095
883 35. R Core Team (2019) R: A Language and Environment for Statistical Computing. R
884 Foundation for Statistical Computing, Vienna, Austria
885 36. Lange E, Tautenhahn R, Neumann S, Gröpl C (2008) Critical assessment of alignment
886 procedures for LC-MS proteomics and metabolomics measurements. BMC
887 Bioinformatics 9:375 . https://doi.org/10.1186/1471-2105-9-375
888 37. Niu W, Knight E, Xia Q, McGarvey BD (2014) Comparative evaluation of eight software
889 programs for alignment of gas chromatography–mass spectrometry chromatograms in
890 metabolomics experiments. Journal of Chromatography A 1374:199–206 .
891 https://doi.org/10.1016/j.chroma.2014.11.005
892 38. Myers OD, Sumner SJ, Li S, Barnes S, Du X (2017) Detailed Investigation and
893 Comparison of the XCMS and MZmine 2 Chromatogram Construction and
894 Chromatographic Peak Detection Methods for Preprocessing Mass Spectrometry
895 Metabolomics Data. Anal Chem 89:8689–8695 .
896 https://doi.org/10.1021/acs.analchem.7b01069
897 39. Hao L, Wang J, Page D, Asthana S, Zetterberg H, Carlsson C, Okonkwo OC, Li L (2018)
898 Comparative Evaluation of MS-based Metabolomics Software and Its Application to

899 Preclinical Alzheimer's Disease. Scientific Reports 8:9291 .
900 https://doi.org/10.1038/s41598-018-27031-x
901 40. Myers OD, Sumner SJ, Li S, Barnes S, Du X (2017) One Step Forward for Reducing False
902 Positive and False Negative Compound Identifications from Mass Spectrometry
903 Metabolomics Data: New Algorithms for Constructing Extracted Ion Chromatograms
904 and Detecting Chromatographic Peaks. Anal Chem 89:8696–8703 .
905 https://doi.org/10.1021/acs.analchem.7b00947
906 41. Schymanski EL, Neumann S (2013) CASMI: And the Winner is . . . Metabolites 3:412–
907 439 . https://doi.org/10.3390/metabo3020412
908 42. Allen F, Pon A, Wilson M, Greiner R, Wishart D (2014) CFM-ID: a web server for
909 annotation, spectrum prediction and metabolite identification from tandem mass
910 spectra. Nucleic Acids Res 42:W94–W99 . https://doi.org/10.1093/nar/gku436
911 43. Allen F, Greiner R, Wishart D (2015) Competitive fragmentation modeling of ESI-
912 MS/MS spectra for putative metabolite identification. Metabolomics 11:98–110 .
913 https://doi.org/10.1007/s11306-014-0676-4
914 44. Loos M (2016) nontarget: Detecting Isotope, Adduct and Homologue Relations in LC-
915 MS Data. https://CRAN.R-project.org/package=nontarget
916 45. Loos M (2016) enviPick: Peak Picking for High Resolution Mass Spectrometry Data.
917 https://CRAN.R-project.org/package=enviPick. Accessed 2 Oct 2018
918 46. Loos M (2018) enviMass version 3.5 LC-HRMS trend detection workflow - R package.
919 https://doi.org/10.5281/zenodo.1213098

47. Meringer M, Reinker S, Zhang J, Muller A MS/MS data improves automated determination of molecular formulas by mass spectrometry. MATCH Commun Math Comput Chem 259–290

48. Ruttkies C, Schymanski EL, Wolf S, Hollender J, Neumann S (2016) MetFrag relaunched: incorporating strategies beyond in silico fragmentation. Journal of Cheminformatics 8:3 . https://doi.org/10.1186/s13321-016-0115-9

49. FOR-IDENT LC. https://water.for-ident.org/#!home. Accessed 7 Feb 2020

50. Tsugawa H, Cajka T, Kind T, Ma Y, Higgins B, Ikeda K, Kanazawa M, VanderGheynst J, Fiehn O, Arita M (2015) MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. Nat Methods 12:523–526 . https://doi.org/10.1038/nmeth.3393

51. Tsugawa H, Kind T, Nakabayashi R, Yukihira D, Tanaka W, Cajka T, Saito K, Fiehn O, Arita M (2016) Hydrogen Rearrangement Rules: Computational MS/MS Fragmentation and Structure Elucidation Using MS-FINDER Software. Anal Chem 88:7946–7958 . https://doi.org/10.1021/acs.analchem.6b00770

52. Röst HL, Sachsenberg T, Aiche S, Bielow C, Weisser H, Aicheler F, Andreotti S, Ehrlich H-C, Gutenbrunner P, Kenar E, Liang X, Nahnsen S, Nilse L, Pfeuffer J, Rosenberger G, Rurik M, Schmitt U, Veit J, Walzer M, Wojnar D, Wolski WE, Schilling O, Choudhary JS, Malmström L, Aebersold R, Reinert K, Kohlbacher O (2016) OpenMS: a flexible open-source software platform for mass spectrometry data analysis. Nature Methods 13:741–748 . https://doi.org/10.1038/nmeth.3959

941 53. Broeckling CD, Afsar FA, Neumann S, Ben-Hur A, Prenni JE (2014) RAMClust: A Novel
942 Feature Clustering Method Enables Spectral-Matching-Based Annotation for
943 Metabolomics Data. Anal Chem 86:6812–6817 . https://doi.org/10.1021/ac501530d
944 54. Böcker S, Letzel MC, Lipták Z, Pervukhin A (2009) SIRIUS: decomposing isotope patterns
945 for metabolite identification. Bioinformatics 25:218–224 .
946 https://doi.org/10.1093/bioinformatics/btn603
947 55. Dührkop K, Shen H, Meusel M, Rousu J, Böcker S (2015) Searching molecular structure
948 databases with tandem mass spectra using CSI:FingerID. PNAS 112:12580–12585 .
949 https://doi.org/10.1073/pnas.1509788112
950 56. Dührkop K, Böcker S (2015) Fragmentation Trees Reloaded. In: Przytycka TM (ed)
951 Research in Computational Molecular Biology. Springer International Publishing, pp
952 65–79
953 57. Böcker S, Dührkop K (2016) Fragmentation trees reloaded. Journal of Cheminformatics
954 8:5 . https://doi.org/10.1186/s13321-016-0116-8
955 58. Dührkop K, Fleischauer M, Ludwig M, Aksenov AA, Melnik AV, Meusel M, Dorrestein
956 PC, Rousu J, Böcker S (2019) SIRIUS 4: a rapid tool for turning tandem mass spectra into
957 metabolite structure information. Nat Methods 16:299–302 .
958 https://doi.org/10.1038/s41592-019-0344-8
959 59. Kuhl C, Tautenhahn R, Böttcher C, Larson TR, Neumann S (2012) CAMERA: An
960 Integrated Strategy for Compound Spectra Extraction and Annotation of Liquid
961 Chromatography/Mass Spectrometry Data Sets. Anal Chem 84:283–289 .
962 https://doi.org/10.1021/ac202450g

963 60. Tautenhahn R, Patti GJ, Rinehart D, Siuzdak G (2012) XCMS Online: A Web-Based
964 Platform to Process Untargeted Metabolomic Data. Anal Chem 84:5035–5039 .
965 https://doi.org/10.1021/ac300698c
966 61. Bruker DataAnalysis. https://www.bruker.com/. Accessed 20 Mar 2020
967 62. Libiseller G, Dvorzak M, Kleb U, Gander E, Eisenberg T, Madeo F, Neumann S,
968 Trausinger G, Sinner F, Pieber T, Magnes C (2015) IPO: a tool for automated
969 optimization of XCMS parameters. BMC Bioinformatics 16:118 .
970 https://doi.org/10.1186/s12859-015-0562-8
971 63. Eliasson M, Rännar S, Madsen R, Donten MA, Marsden-Edwards E, Moritz T, Shockcor
972 JP, Johansson E, Trygg J (2012) Strategy for Optimizing LC-MS Data Processing in
973 Metabolomics: A Design of Experiments Approach. Anal Chem 84:6869–6876 .
974 https://doi.org/10.1021/ac301482k
975 64. Loos M, Singer H (2017) Nontargeted homologue series extraction from hyphenated
976 high resolution mass spectrometry data. J Cheminform 9:12 .
977 https://doi.org/10.1186/s13321-017-0197-z
978 65. Schollée JE, Bourgin M, von Gunten U, McArdell CS, Hollender J (2018) Non-target
979 screening to trace ozonation transformation products in a wastewater treatment train
980 including different post-treatments. Water Research 142:267–278 .
981 https://doi.org/10.1016/j.watres.2018.05.045
982 66. Csardi G, Nepusz T (2006) The igraph software package for complex network research.
983 InterJournal Complex Systems:1695
984 67. Almende B.V., Thieurmel B, Robert T (2019) visNetwork: Network Visualization using
985 “vis.js” Library. https://CRAN.R-project.org/package=visNetwork

986 68. Kujawinski EB, Behn MD (2006) Automated Analysis of Electrospray Ionization Fourier
987 Transform Ion Cyclotron Resonance Mass Spectra of Natural Organic Matter. Anal
988 Chem 78:4363–4373 . https://doi.org/10.1021/ac0600306
989 69. Koch BP, Dittmar T (2006) From mass to structure: an aromaticity index for high-
990 resolution mass data of natural organic matter. Rapid Communications in Mass
991 Spectrometry 20:926–932 . https://doi.org/10.1002/rcm.2386
992 70. Koch BP, Dittmar T (2016) From mass to structure: an aromaticity index for high-
993 resolution mass data of natural organic matter. Rapid Communications in Mass
994 Spectrometry 30:250–250 . https://doi.org/10.1002/rcm.7433
995 71. Brock O, Helmus R, Kalbitz K, Jansen B Non-target screening of leaf litter-derived
996 dissolved organic matter using liquid chromatography coupled to high-resolution mass
997 spectrometry (LC-QTOF-MS). European Journal of Soil Science.
998 https://doi.org/10.1111/ejss.12894
999 72. Heller SR, McNaught A, Pletnev I, Stein S, Tchekhovskoi D (2015) InChI, the IUPAC
1000 International Chemical Identifier. Journal of Cheminformatics 7:23 .
1001 https://doi.org/10.1186/s13321-015-0068-4
1002 73. Guha R (2007) Chemical Informatics Functionality in R. Journal of Statistical Software
1003 18:1–16
1004 74. Schymanski EL, Gerlich M, Ruttkies C, Neumann S (2014) Solving CASMI 2013 with
1005 MetFrag, MetFusion and MOLGEN-MS/MS. Mass Spectrometry 3:S0036–S0036 .
1006 https://doi.org/10.5702/massspectrometry.S0036

75. Langfelder P, Zhang B (2016) dynamicTreeCut: Methods for Detection of Clusters in Hierarchical Clustering Dendrograms. https://CRAN.R-project.org/package=dynamicTreeCut
76. Royal Society of Chemistry ChemSpider. http://www.chemspider.com. Accessed 6 Feb 2020
77. Lex A, Gehlenborg N, Strobelt H, Vuillemot R, Pfister H (2014) UpSet: Visualization of Intersecting Sets. IEEE Transactions on Visualization and Computer Graphics 20:1983–1992 . https://doi.org/10.1109/TVCG.2014.2346248
78. Chen H, Boutros PC (2011) VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. BMC Bioinformatics 12:35 . https://doi.org/10.1186/1471-2105-12-35
79. Gu Z, Gu L, Eils R, Schlesner M, Brors B (2014) circlize implements and enhances circular visualization in R. Bioinformatics 30:2811–2812
80. Gehlenborg N (2019) UpSetR: A More Scalable Alternative to Venn and Euler Diagrams for Visualizing Intersecting Sets. https://CRAN.R-project.org/package=UpSetR
81. Xie Y, Allaire JJ, Grolemund G (2018) R Markdown: The Definitive Guide. Chapman and Hall/CRC, Boca Raton, Florida
82. Allaire JJ, Xie Y, McPherson J, Luraschi J, Ushey K, Atkins A, Wickham H, Cheng J, Chang W, Iannone R (2019) rmarkdown: Dynamic Documents for R
83. Iannone R, Allaire JJ, Borges B (2018) flexdashboard: R Markdown Format for Flexible Dashboards. https://CRAN.R-project.org/package=flexdashboard
84. Chang W, Cheng J, Allaire JJ, Xie Y, McPherson J (2019) shiny: Web Application Framework for R. https://CRAN.R-project.org/package=shiny

85. Helmus R (2020) patRoon 1.0.0 manuals. Zenodo. https://doi.org/10.5281/zenodo.3889937
86. patRoon reference. https://rickhelmus.github.io/patRoon/reference/index.html. Accessed 11 Jun 2020
87. patRoon tutorial. https://rickhelmus.github.io/patRoon/articles/tutorial.html. Accessed 11 Jun 2020
88. Helmus R patRoon handbook. https://rickhelmus.github.io/patRoon/handbook_bd/index.html. Accessed 11 Jun 2020
89. Xie Y (2016) bookdown: Authoring Books and Technical Documents with R Markdown. Chapman and Hall/CRC, Boca Raton, Florida
90. Xie Y (2019) bookdown: Authoring Books and Technical Documents with R Markdown
91. Wickham H, Danenberg P, Csárdi G, Eugster M (2019) roxygen2: In-Line Documentation for R. https://CRAN.R-project.org/package=roxygen2
92. Helmus R (2020) patRoonData. https://github.com/rickhelmus/patRoonData. Accessed 18 Mar 2020
93. Helmus R, Albergamo V (2020) patRoonData: 1.0.0. Zenodo. https://doi.org/10.5281/zenodo.3743266
94. Lang M (2017) checkmate: Fast Argument Checks for Defensive R Programming. The R Journal 9:437–445
95. Csárdi G, Chang W (2019) processx: Execute and Control System Processes. https://CRAN.R-project.org/package=processx
96. R Special Interest Group on Databases (R-SIG-DB), Wickham H, Müller K (2019) DBI: R Database Interface. https://CRAN.R-project.org/package=DBI

97. Müller K, Wickham H, James DA, Falcon S (2019) RSQLite: “SQLite” Interface for R. https://CRAN.R-project.org/package=RSQLite
98. Eddelbuettel D, François R (2011) Rcpp: Seamless R and C++ Integration. Journal of Statistical Software 40:1–18 . https://doi.org/10.18637/jss.v040.i08
99. Eddelbuettel D (2013) Seamless R and C++ Integration with Rcpp. Springer, New York
100. Eddelbuettel D, Balamuta JJ (2017) Extending R with C++: A Brief Introduction to Rcpp. PeerJ Preprints 5:e3188v1 . https://doi.org/10.7287/peerj.preprints.3188v1
101. Kapoulkine A pugixml. https://pugixml.org/. Accessed 6 Feb 2020
102. Dowle M, Srinivasan A (2019) data.table: Extension of `data.frame`. https://CRAN.R-project.org/package=data.table
103. MetFragR. http://ipb-halle.github.io/MetFrag/projects/metfragr/. Accessed 6 Feb 2020
104. Lang DT (2019) RDCOMClient: R-DCOM client
105. Wickham H (2011) testthat: Get Started with Testing. The R Journal 3:5–10
106. Henry L, Sutherland C, Hong D, Luciani TJ, Decorde M, Lise V (2019) vdiffr: Visual Regression Testing and Graphical Diffing. https://CRAN.R-project.org/package=vdiffr
107. Helmus R (2020) patRoon benchmarking & demonstration data. Zenodo. https://doi.org/10.5281/zenodo.3885448
108. Mersmann O (2019) microbenchmark: Accurate Timing Functions. https://CRAN.R-project.org/package=microbenchmark
109. Fischer B, Neumann S, Gatto L, Kou Q, Rainer J (2020) mzR: parser for netCDF, mzXML, mzData and mzML and mzIdentML files (mass spectrometry data). https://bioconductor.org/packages/mzR/. Accessed 6 Apr 2020
110. Gurobi. https://www.gurobi.com/. Accessed 6 Feb 2020

111. CPLEX Optimizer. https://www.ibm.com/analytics/cplex-optimizer. Accessed 6 Feb 2020
112. GNU Project - Free Software Foundation (FSF) GLPK (GNU Linear Programming Kit). https://www.gnu.org/software/glpk/. Accessed 6 Feb 2020
113. Böcker S, Dührkop K, Fleischauer M, Ludwig M (2019) SIRIUS Documentation Release 4.0.1
114. NORMAN Suspect List Exchange – NORMAN SLE. https://www.norman-network.com/nds/SLE/. Accessed 13 Mar 2020
115. CompTox March 2019 CSV file. ftp://newftp.epa.gov/COMPTOX/Sustainable_Chemistry_Data/Chemistry_Dashboard/MetFrag_metadata_files/CompTox_17March2019_SelectMetaData.csv
116. Djoumbou-Feunang Y, Fiamoncini J, Gil-de-la-Fuente A, Greiner R, Manach C, Wishart DS (2019) BioTransformer: a comprehensive computational tool for small molecule metabolism prediction and metabolite identification. Journal of Cheminformatics 11:2 . https://doi.org/10.1186/s13321-018-0324-5
117. Kruve A (2019) Semi-quantitative non-target analysis of water with liquid chromatography/high-resolution mass spectrometry: How far are we? Rapid Communications in Mass Spectrometry 33:54–63 . https://doi.org/10.1002/rcm.8208
118. Djoumbou Feunang Y, Eisner R, Knox C, Chepelev L, Hastings J, Owen G, Fahy E, Steinbeck C, Subramanian S, Bolton E, Greiner R, Wishart DS (2016) ClassyFire: automated chemical classification with a comprehensive, computable taxonomy. J Cheminform 8:61 . https://doi.org/10.1186/s13321-016-0174-y
119. R for Mass Spectrometry. www.rformassspectrometry.org. Accessed 13 Mar 2020

120. Rick Helmus (2020) patRoon 1.0.0. Zenodo. https://doi.org/10.5281/zenodo.3889856

Figures

Figure 1. Generic workflow for environmental non-target analysis.

Figure 2. Overview of the NTA *patRoon* workflow. All steps are optional. Steps that are connected by blue and straight arrows represent a one-way data dependency, whereas steps connected with red curved and dashed arrows represent steps with two-way data interaction.

Figure 3. Graphical user interface tools in *patRoon*. Tools are provided (a) to create a new *patRoon* data analysis project and (b) to inspect feature chromatography data.

Figure 4. Interface for the *patRoon* workflow. The workflow steps are performed by a set of functions that execute the selected algorithm and return the data in a harmonized format by utilizing the 'S4' object oriented programming approach of *R*. These objects all derive from a common base class and may be further sub-classed in algorithm specific classes (as is exemplified for `features`). Generic functions are defined for all workflow classes to implement further data processing functionality in a predictable and algorithm independent manner (see also Table 3). Further information is provided in the reference manual [85, 86].

Figure 5. Parallelization benchmark results. (a) Benchmark results for commonly used CLI tools applied in *patRoon* workflows under varying parallelization conditions. Tests were

performed with “simple” (left) and “complex” (right) input conditions (Table 4) to simulate varying workflow complexity. Parallelization was performed with the multiprocessing functionality of *patRoon* (top) or by using native multithreading (bottom, for tools that supported this). Graphs represent number of processes or threads versus relative execution time (normalized to sequential results). The dotted grey lines represent the theoretical trend if maximum parallelization performance is achieved. The dashed blue line represents the number of physical cores that became the default selection in *patRoon* based on these results. (b) Comparison of execution times (normalized to the execution times of the unoptimized results) when tools are executed without optimizations (green), executed with native multithreading (FFM, SIR and MF) or batch mode (GF) (orange), executed with multiprocessing (purple) or a combination of the latter two (pink), using simple (left) and complex (right) input conditions. (c) Overview of execution times for a complete *patRoon* workflow executed under optimized versus unoptimized conditions. All results for MC and SIR were obtained without enabling their native batch mode.

Figure 6. Common visualization functionality of *patRoon* applied to the demonstrated workflow. From left to right: an *m/z vs* retention time plot of all feature groups, an EIC for the tramadol suspect found in both influent samples, a compound annotated spectrum for the 1,2,3-benzotriazole suspect and comparison of feature presence between sample groups using UpSet [77], Venn and chord diagrams.

Supplementary information

Additional file 1: Comma-separated file (.csv). Overview of software and databases that are used in the implementation in *patRoon*. This table summarizes all the software and databases that are described in the implementation section of the main text.

Additional file 2: Word document (.docx). Supplementary figures. Additional figures that illustrate implementation details of *patRoon* and miscellaneous benchmarking results.

Additional file 3: Word document (.docx). Supplementary tables. Additional tables with more details on the implementation and suspect screening demonstration.

Additional file 4: Zip archive (.zip). Source code for benchmarks. Archive with several *R* scripts that were used to perform the parallelization benchmarks.

Additional file 5: Comma-separated file (.csv). Demonstration suspect list. Suspect list that was used for the *patRoon* demonstration. The list was based on the detected compounds reported in [11], and SMILES identifiers for each suspect were collected from PubChem [23].

		HRMS data	Features				Annotation								Primary Interface	Language	OS	License	References
		PP	FTS	FG	C	SUS	MS	FA	CA	LA	HS	GA	C	RT					
a	*CFM-ID*								X	X					CLI, Web	C++	Cross	LGPLv2.1	[42, 43]
b	*enviMass, enviPick, nontarget*	X[i]	**X**	X	X	X					**X**	X			GUI, R, Web	R	Cross	GPLv3.0[1]	[44–46]
c	*GenForm*							**X**							CLI	C++	Cross[2]	LGPLv2.0	[47]
d	*MetFrag*								**X**	**X**			X	X	CLI, R, Web	Java	Cross	LGPLv2.0	[48]
e	*FOR-IDENT*								X[d]	X				X	Web	HTML	Cross	Closed	[49]
f	*MS-DIAL, MS-FINDER*		X	X	X	X	X	X	X	X		X			CLI, GUI	C#	Win	LGPLv3.0	[50, 51]
g	*MZmine*	X	X[gl]	X	X	X	X	X	X[k]	X		X[gl]			GUI	Java	Cross	GPLv2.0	[33]
h	*OpenMS*	**X**[hi]	**X**	**X**			X		X[k]	X		X			CLI, GUI, Python	C++	Win, Lin, Mac	BSD/3-Clause	[52]
i	*ProteoWizard*	**X**													CLI, GUI	C++	Win, Lin	Apache 2.0	[22]
j	*RAMClustR*						X					**X**			R	R	Cross	GPLv2.0	[53]
k	*SIRIUS and CSI:FingerID*							**X**	**X**				X		CLI, GUI	Java	Cross	GPLv3.0	[54–58]
l	*XCMS and CAMERA*		**X**	**X**	X							**X**			R	R	Cross	GPLv2.0	[32, 59]
m	*XCMS Online*	X	X[l]	X[l]			X			X		X			Web	R	Cross	Closed	[60]
n	*patRoon*	**X**[hi]	**X**[bhl]	**X**[hl]	**X**	**X**	**X**	**X**[ck]	**X**[dk]	**X**[d]	**X**[b]	**X**[jl]	**X**	**X**[d]	R	R	Cross	GPLv3.0	

P: pre-processing; FTS: find features; FG: group features across samples; C: data clean up; SUS: suspect screening; MS: automatic MS data extraction for annotation purposes; FA: formula annotation; CA/LA: compound annotation (*in silico*/library); HS: unsupervised homologous series extraction; GA: grouping and annotating chemically related features (e.g. adducts, isotopes, in-source fragments); RT: retention time prediction; Bold: functionality integrated in *patRoon*; superscript: implemented with algorithms by given rows (omitted if only native); CLI: command-line interface; GUI: graphical user interface; Web: interfaced via internet browser; OS: Supported Operating Systems; Win: *Microsoft Windows*; (Lin): GNU/Linux, (Mac): macOS; Cross: cross-platform; (1): enviMass is distributed commercially; (2): Only *Microsoft Windows* binaries are distributed.