analytical chemistry

pubs.acs.org/ac Article

Best Practices in GC−MS and GC × GC−MS-Based Metabolomics and Volatile Analyses: An International Survey

Ryland T. Giebelhaus, Michael Herold, James J. Harynuk, David E. Alonso, Bashar Amer, Corey D. Broeckling, Pantelis Charisiadis, Raquel Cumeras, A. Paulina de la Mata, Warwick Dunn, Flavio A. Franchina, Helen Gika, Jennifer A. Kirwan, Isabella H. Muti, Lukáš Najdekr, Estelle Pujos-Guillot, Pierre-Hugues Stefanuto, Teresa Steininger-Mairinger, Alpesh Thakker, and Oliver Fiehn*

Cite This: https://doi.org/10.1021/acs.analchem.5c06918 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Standardized quality assurance and quality control (QA/QC) practices are essential for reproducible GC−MS metabolomics, yet systematic documentation of current laboratory practices has been lacking. Here, as part of the Metabolomic Quality Assurance and Quality Control Consortium (mQACC), we surveyed 85 laboratories from 27 countries to characterize QA/QC implementation and establish evidence-based recommendations. Respondents represented diverse applications, with 79% performing untargeted analysis, 60% conducting targeted analyses, and 44% conducting both. While single column chromatography is clearly the norm, 24% of the participants used multidimensional chromatography to improve the separation of complex mixtures. Electron ionization with autotuning dominated >95% of the respondents, but more than 30% of the laboratories at least occasionally also used chemical ionization. While most laboratories used low-resolution mass spectrometers, almost half of the laboratories also performed GC−MS analyses on high-resolution QTOF or Orbitrap instruments. A strong consensus emerged on critical QA/QC practices: >90% of laboratories use internal standards for quality control, perform regular leak checks, and maintain injector systems through routine component replacement, spanning column (exchange/cuts), liners, syringes, and septa. Routine monitoring (>50%) involves method blanks, peak shape assessments, and systematic evaluation of intensity drifts, carryovers, and contamination. Retention indices coupled with mass spectral library matching served as the primary annotation approach (60%). Overall, a consensus of best practices in QA/QC and reporting emerged, providing evidence-based recommendations for high-quality GC−MS metabolomics.

INTRODUCTION

Gas chromatography−mass spectrometry (GC−MS) is a widely utilized analytical technique for the qualitative and quantitative analysis of complex chemical mixtures.[1,2] Its applications span numerous fields, including metabolomics,[3] environmental analysis,[4] food safety,[5] and clinical research.[6] GC−MS enables the separation and identification of volatile and semivolatile compounds with high sensitivity and specificity.[7] Given its versatility, researchers and laboratories worldwide employ GC−MS for diverse purposes, such as targeted quantitative analysis, untargeted metabolomic profiling, and isotope enrichment studies.[8] Proper quality management in GC−MS metabolomics is essential to ensure reproducibility, reliability, and fidelity in the data.[9,10] According to ISO9000 (2015), quality assurance (QA) encompasses the proactive activities a laboratory implements to ensure that quality requirements are met prior to analysis, whereas quality control (QC) refers to the reactive processes used to verify and document that those requirements have been achieved.[10,11] Generally, QA is applied before data acquisition, and QC is applied during and after.[9] Examples of QA include standardized training, instrument system suitability tests, leak tests, calibration, and standard operating procedures.[9,10] QC measurements include analysis of reference standards, pooled samples, and blanks.[9,10,12,13] These definitions for QA and QC are being used by the Metabolomics Quality Assurance and Quality Control Consortium (mQACC).[9]

Here, a detailed survey was conducted among researchers and practitioners actively employing this technology to understand the current practices, trends, and challenges associated with QA/QC in GC−MS workflows. The questionnaire captured responses across various aspects,

Received: November 3, 2025
Revised: April 18, 2026
Accepted: April 21, 2026

including project frequencies, types of analyses, quality control measures, and maintenance practices. Specifically, the survey sought to address the following questions:

1. Frequency of GC−MS usage: How many projects are conducted annually, and what proportion are metabolomics-related?
2. Purpose of analyses: What are the primary objectives for using GC−MS, such as targeted analysis, untargeted screening, or isotope enrichment?
3. Quality control measures: What QA/QC measures and validations are employed to ensure data reliability and reproducibility?
4. Maintenance practices: How frequently are routine and specialized maintenance activities performed on GC−MS systems as part of QA practices?
5. Reporting standards: How do researchers report metabolomics standards (e.g., Metabolomics Standards Initiative levels) and confidence in compound identification?

Here, we discuss the questionnaire results and their implications for QA/QC, and we provide general recommendations for performing targeted and untargeted GC−MS metabolomics (trends and perspectives).

EXPERIMENTAL SECTION

Survey Administration

Members and nonmembers of the mQACC consortium who utilize GC−MS technologies (including multidimensional GC−MS) for metabolomics were asked to voluntarily respond to a questionnaire, providing the QA and QC practices utilized in their laboratory. Each participant completed the survey questions (Supporting Information Section 1) provided through Google Forms. We welcomed participants in all positions within their laboratories to respond to the survey. The positions of each respondent were not collected.

The survey consisted of over 40 questions divided into five major sections, where users were asked to discuss the frequency of use, maintenance practices, and QA/QC procedures for each section: (1) general aspects to gauge the scope and frequency of GC−MS use; (2) sample preparation methods, sample introduction, and injection systems; (3) chromatographic parameters; (4) mass spectrometry use and maintenance; and (5) data processing procedures. For each section, a combination of multiple-choice questions and open comment fields was provided to determine the frequency and purpose of each practice.

Users were required to answer all questions except for optional open comment fields, which were provided after questions where users could respond with "Other (specify below)". Open comment field responses were summarized manually. All data were anonymized and exported into a single Excel spreadsheet for analysis.

Data Processing and Analysis

Data were initially analyzed in the Google Forms user interface, utilizing the plots and figures it produces based on the survey results. Data were then exported to an Excel table, where any identifying information was removed before data were shared among the GC−MS working group of mQACC. Additionally, ChatGPT 4o was used to summarize textual answers and data analyses when required. All results from ChatGPT were manually inspected for accuracy. ChatGPT 4o did not receive any identifying information and did not participate in the curation of this publication.

RESULTS AND DISCUSSION

Respondent Demographics

Eighty-five (N = 85) respondents chose to answer the questionnaire and participate. All responses were completed fully. Participants were from 27 countries (Table S1) including respondents from every continent excluding Antarctica (Figure 1), with most respondents from Europe (N = 33, 39%) and

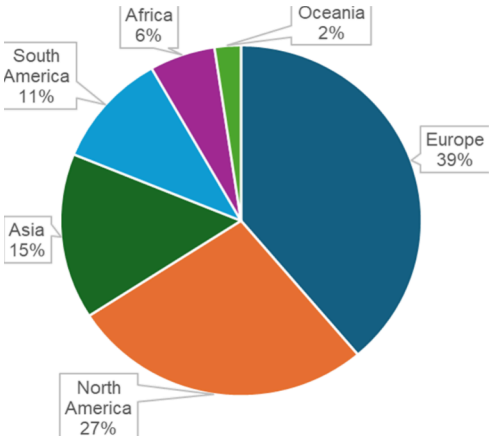

Figure 1. Breakdown of respondents by continent.

North America (N = 23, 27%). We found it challenging to circulate the survey in Asia, particularly China, and therefore, these research groups are likely underrepresented. We speculate that the survey did not reach researchers in China as access to LinkedIn (platform used to advertise the survey) and Google products including Forms is severely limited.[14] Among respondents, the vast majority were in academic, hospital, or government laboratories, with some respondents coming from industry. The respondents' email domain was used to make this determination and therefore may not be entirely accurate. Absolute values for this metric were not computed. Deidentified data were compiled into an Excel file available with the Supporting Information.

Typical Use of GC−MS-Based Metabolomics

When gauging GC−MS use (Q-1.1), irrespective of the analysis purpose, most respondents (43.5%) stated that they perform more than two GC−MS projects per year, with 18.8% reporting over 50 projects per year or almost daily use. The term "project" was not specified in the questionnaire in relation to the number of samples, and it can mean a small batch or multiple-batch project over many days. Over half (56.5%) of respondents reported less than 10 projects per year with GC−MS. When asked how many metabolomics projects participants perform per year (Q-1.2), irrespective of the analytical technique, the bulk of respondents stated that they performed metabolomics, with few (11.8%) reporting they never performed metabolomics-type analyses. Additionally, the same number of respondents in Q-1.1 reported performing one to two projects per year as responding in Q-1.2 and not performing metabolomics. These respondents were not correlated, with only two respondents reporting across both Q-1.1 and Q-1.2.

In Q-1.3, 15.3% of respondents reported that none of their metabolomics projects use GC−MS. All respondents from Q-1.2 that reported not using metabolomics also reported that they did not use GC−MS for metabolomics, which was expected. Outliers stated that they performed >50 projects per year while reporting no use of GC−MS for metabolomics.

Overall, our survey captures a wide range of use habits for GC−MS and metabolomics, such as core facilities, rather than sampling only heavy users of GC−MS metabolomics.

https://doi.org/10.1021/acs.analchem.5c06918
Anal. Chem. XXXX, XXX, XXX−XXX

Purpose of GC−MS Metabolomics Analyses

In Q-1.4, the bulk of respondents stated that they are performing untargeted metabolomics (N = 67, 78.8%), with over half (60%) of respondents also stating that they perform targeted metabolomics, with one respondent stating that they perform targeted analysis but without quantification. Additionally, almost half (43.5%) reported performing both targeted and untargeted studies (Figure 2). Isotopic enrichment studies were less popular (17.6%). A large portion of respondents (29.4%) stated that they performed both untargeted and targeted metabolomics. Most users of isotopic enrichment (12.9%) reported performing both untargeted and targeted analyses, likely because these users of isotopic enrichment are simultaneously performing untargeted or targeted analyses of their samples in these studies. The distributions of the number of projects performed by untargeted and targeted were very similar, with the modal class being 2 to 10 projects per year in both cases. The diverse use cases in the survey reflect trends in the GC−MS community.

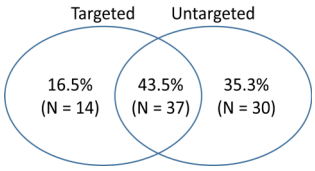

Figure 2. Use of targeted, untargeted, and both by respondents. Targeted analysis is independent of whether quantification was performed or not.

As was expected, most respondents (74.1%) were using 1D GC−MS exclusively over multidimensional GC−MS (including GC−GC and GC × GC). Twenty percent of respondents reported using both 1D GC and multidimensional GC, with three respondents reporting only using multidimensional GC. Some respondents reported using neither method, potentially not understanding the difference between the techniques.

Multidimensional GC techniques provide significantly more resolution, sensitivity, and peak capacity, making them ideal for metabolomics analyses. However, the added second dimension of separation requires additional hardware and an experienced user to operate and maintain the instrument. Additionally, the data are considerably more complex to analyze. All of these reasons contribute to the metabolomics community currently preferring GC−MS over multidimensional GC.

GC−MS Instrument Configuration

Most respondents reported performing liquid injections with or without derivatization. Slightly more use cases were reported for splitless injections (84.7%) compared with split injections (78.8%), with very few respondents reporting other liquid injections. About half of the respondents did not perform volatile analysis. Of those who report volatile analysis, solid phase microextraction (SPME) is slightly more popular than headspace (including static and dynamic). This was interesting, as one significant advantage of GC−MS over LC−MS is its ability to detect volatile species. However, many researchers in the literature report using GC−MS with derivatization to detect polar and semipolar metabolites. Trimethylsilylation is the most popular derivatization reaction in the literature and, unsurprisingly, was the most popular in this survey. Liners with wool were by far the most popular (84.7%).[2,15]

Guard columns were surprisingly unpopular, with most respondents (61%) reporting not using one. Guard columns protect the analytical column, extending the column life. This is particularly useful when analyzing complex biological samples by liquid injection. Band broadening can occur with guard columns, slightly reducing the sensitivity. Keeping guard columns short and using low-dead volume connectors such as SilTite can prevent leaks and further band broadening. We recommend the use of a guard column to increase column life (or using an uncoated capillary as a retention gap) when analyzing complex samples. However, each analyst needs to perform method validation to determine if a guard column or retention gap is fit for purpose and the analytical trade-offs are acceptable.[16,17] Any shifts in retention time are corrected when using retention indices.[2]

By far, the most common column chemistry for one-dimensional GC analyses was the ubiquitous "-5" phase (5% phenyl, 95% dimethylpolysiloxane (or polysylphenylene oxide)) used by 66.7% of respondents. Polyethylene glycol (wax)-type phases were the second most popular (13.5%). Less popular are pure PDMS-type ("-1") phases, low cyano phases (-1701 and -624 types), and high-phenyl phases (50% or 35% phenyl) (5%). There were also a handful of specialty phases reportedly in use (70% cyano, cyclodextrin, proprietary dioxin phases, and HT-8).

Presumably, there are several reasons that contribute to the popularity of the "-5" phases including the general robustness and stability of the phases and the availability of retention index libraries to aid in compound identification. For these reasons, when developing a new method, we recommend starting with a "-5" phase if no similar methods exist in the literature. These columns are ubiquitous, and vendors often provide them with new instruments.

For GC × GC separations, most users reported using a "-5" column in the first dimension followed by 50% phenyl, wax, or trifluoropropyl column. A second pair of column sets that were reported by some users was a -624 column in 1D followed by a wax in 2D, or vice versa. For developing new GC × GC methods, we suggest starting with a "-5" phase in 1D and a 50% phenyl in 2D if no similar methods are reported in the literature.

For those users who reported column dimensions, most columns for 1D GC and the first dimension of a GC × GC separation were 25−30 m in length with 0.25 mm internal diameters and 0.25 μm film thickness. A small number of users reported using either shorter (10−15 m) or longer (60 m) columns or 0.18 mm internal diameter columns.

Quadrupole systems, both single and triple quadrupole, were the most common (41.1%) followed by time-of-flight MS (TOFMS; 27%) and Orbitrap systems (9.4%). Most targeted work was performed on a quadrupole (Figure 3). Interestingly, almost half of the untargeted work was also performed on a quadrupole MS system (25.8%). Other studies have similarly reported that quadrupoles are the most common for GC−MS metabolomics[18,19] Time-of-flight systems were almost exclusively used for untargeted analysis. Other respondents reported using cyclic ion mobility quadrupole TOFMS, ion trapping, and high-resolution magnetic sector instruments.

https://doi.org/10.1021/acs.analchem.5c06918
Anal. Chem. XXXX, XXX, XXX−XXX

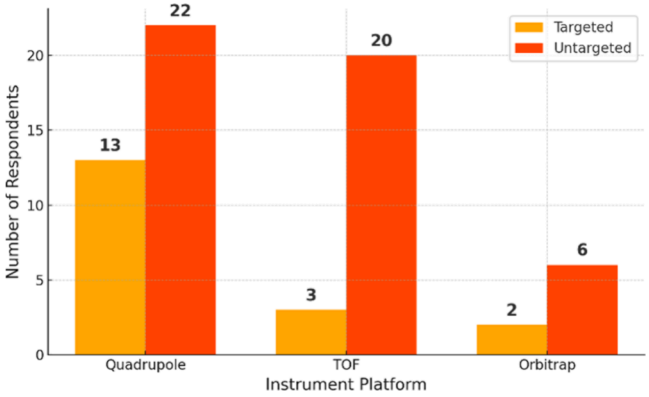

Figure 3. Reported mass spectrometers used by analysis type, targeted or untargeted. Quadrupole includes both single and triple quadrupole systems.

QA and QC General Practices

The overwhelming majority of respondents reported performing QC measures and validations, demonstrating that the GC−MS metabolomics community is concerned with ensuring rigorous data quality. The majority reported evaluating the peak shape, ratio of peak intensities from samples to blanks, effect of sample matrix on peak intensities, effect of run sequence on peak intensities as QC measures, and validations they use to indicate potential performance issues in their analyses and trigger preventative maintenance interventions. Only two respondents reported performing no QC. One respondent, who also performs forensic analysis, reported using comprehensive control samples to monitor every step of the full analytical method, likely motivated by legal requirements. In specific cases, forensic chemists may be hesitant to spike standards into samples for QA/QC despite community consensus in other fields of GC−MS analysis. Metabolomics QA/QC requirements will vary between fields, such as forensics, clinical screening, and agriculture, and depend on whether the analysis is targeted or untargeted and the existence of standard criteria for certain applications (e.g., toxicological screening).

Other QA practices were performed by many participants, such as checking the effect of the injection temperature and volume on peak intensity and optimizing the splitless time. In the long-form responses, participants largely stated that they performed internal standard spiking for QC purposes with both native and isotopically labeled standards. Some users also reported monitoring peak shape and intensity using standard solution mixes, including the Grob test mixture or custom in-house mixes.[20,21] At a minimum, we suggest that users monitor blanks (reagent and instrument) for background peaks and monitor peak shape and intensity using a standard solution mix.

Maintenance Practices

Almost all respondents reported performing maintenance on the GC injection system. Liner exchange, O-ring exchange, septum exchange, and syringe exchange were almost unanimously performed at least once annually but much more frequently by many respondents. More in-depth maintenance such as split vent trap exchange or gas line cleanup was performed less often.

Most respondents stated that they exchanged or cut the column at least once annually. Additionally, almost all respondents performed regular leak checks. Numerous respondents reported that baking out the column or replacing the guard column was part of column maintenance. For both injector system and column maintenance, we suggest using QA/QC results (e.g., blanks and peak shape) to inform when maintenance should be performed. Reasons for prompt maintenance are detailed in the next section.

On the MS systems, ion source cleaning, exchanging filaments, and changing pump oil were most performed, with almost all respondents reporting these within the past year. More involved maintenance such as transfer line cleaning and detector exchange were performed less frequently. One respondent stated that they checked the turbo pump function and power, presumably using the software and power consumption readings. Most vendors provide guidance on when to change pump oil and provide ranges for the turbo pump function. QA/QC can inform decisions, such as when to clean the ion source or replace the detector.

Major Reasons Prompting GC−MS Maintenance

GC−MS users implement preventive maintenance (Q-3.5) through a combination of proactive strategies and reactive adjustments to ensure the instrument reliability and data accuracy. The main reasons have been classified into five categories:

- Performance issues (64.7%) include loss of signal intensity/sensitivity (17.6%), poor peak shapes (21.1%), reduced resolution (8.2%), retention time shifts (5.9%), and increased background noise (11.8%).
- Scheduled maintenance (48.2%) involves regular preventive upkeep (16.5%), postinjection protocols (5.9%), transitions between studies/users (3.5%), and calendar-based tasks (5.9%).
- Calibration and testing were prompted by calibration/tuning deviations (7.1%), alongside instrument-specific adjustments like MS parameter changes (3.5%) and leak checks (3.5%).
- System suitability tests focus on QC deviations, reported by 37.6% of respondents, such as failed QC samples (12.9%), abnormal blanks (3.5%), internal standard issues (4.7%), and mass/fragmentation anomaly detection (2.4%).
- Other factors were cited by 23.5% of respondents and include sample-related challenges like column overloading (2.4%) and contamination (4.7%) as well as proactive measures to enhance functionality (5.9%), extend performance (2.4%), and maintain data quality (8.2%). Overall, maintenance balances structured preventive protocols with reactive troubleshooting to optimize GC−MS performance and uphold data integrity.

The frequency and type of major reason will depend on the analyses the user is performing. For example, analyzing complex liquid samples, such as derivatized urine samples, in splitless mode will contaminate and degrade the column's performance much faster than analyzing volatile samples by SPME using a high split. While analysts should be aware of all major reasons to prompt GC−MS maintenance, paying attention to sample-specific issues will ensure that analysis is performed with appropriate QA/QC.

Data Processing: GC−MS-Based Metabolomics Reporting

Reporting standards in metabolomics analyses show highly congruent practices, with specific variations for targeted analysis use cases. Retention time drifts are usually accounted for by retention index calculations, using temperature

https://doi.org/10.1021/acs.analchem.5c06918
Anal. Chem. XXXX, XXX, XXX−XXX

programming retention indices from a series of homologous compounds (e.g., *n*-alkanes or fatty acid methyl esters (FAMES)).[22,23] Each of these techniques relies on the high reproducibility of retention times in gas chromatography. In comparison, retention time locking is used by less than half of all respondents in at least one project per year, for which an internal standard is used to change data acquisition parameters such as the helium gas flow. For GC × GC separations, users also reported adding a custom standard mix to monitor 2D retention times to indicate when the 2D column needs to be changed. We strongly recommend that all practitioners of GC−MS use some technique to account for retention time drift, the easiest being using retention indices computed from a standard mixture. Most commercial mass spectral libraries include temperature-program retention indices on a "-5" column.

Interestingly, differences between data processing methods are not a prominent concern for GC−MS practitioners. Unlike for (nontargeted) LC−MS/MS, there are very few research papers on the impact of software use in GC−MS. We hypothesize that this fact has three major reasons: (1) GC−MS ionization usually does not suffer much from ion suppression as is seen in electrospray ionization (ESI); (2) LC−MS often shows spray heterogeneity,[24] leading to ragged extracted ion chromatograms, unlike GC−MS peaks; and (3) as GC−MS typically uses electron ionization, all spectra have rich fragment information, which is useful for MS deconvolution of coeluting compounds. Peak deconvolution has been successfully established in open-source software (AMDIS[25] and MS-DIAL[26]) as well as commercial software (LECO ChromaTOF, Thermo TraceFinder) for over 20 years. For GC × GC-TOFMS analyses, scan speeds are typically 150−200 Hz to yield good deconvolution for peak widths of 50−100 ms. In addition, the use of targeted analytical chemistry in GC−MS has been practiced for more than 60 years with dedicated established workflows in data processing software. The use of specific software used by respondents was not collected and should be the subject of another community-wide survey.

We initially hypothesized that practitioners of untargeted GC−MS metabolomics would predominantly use TOFMS or Orbitrap-style MS systems as they are well suited for untargeted analysis. Surprisingly, we found that 22 respondents used quadrupole systems (single and triple quadrupoles were not differentiated in this survey) for untargeted analysis versus 20 respondents using a TOFMS and 6 respondents using an Orbitrap MS instrument for untargeted analysis. As was expected, quadrupole style instruments were overwhelmingly reported as the choice of detector for targeted studies. These results were computed from respondents exclusively reporting performing untargeted or targeted analysis but not both. We recommend that practitioners of untargeted metabolomics use mass spectrometers with fast data acquisitions whenever possible to maximize data fidelity and mass spectral quality.[27] Quadrupole MS systems are slower than TOFMS instruments in data acquisition, scanning one ion at a time.[28] Hence, quadrupole systems may undersample narrow peaks, especially for MS deconvolution of coeluting chromatographic peaks. In contrast, fast data acquisition instruments such as TOFMS provide spectral continuity, where ion packets are formed in the source, extracted, and analyzed almost immediately. Additionally, wherever possible, we recommend that analysts use MS systems with mass resolution and accuracy to further increase confidence when assigning putative identities to metabolites. Nominal mass is often insufficient for reliably identifying fragments, particularly in cases where there are numerous heteroatoms on the metabolites.[29] Accurate mass serves to eliminate both false positives and false negatives in untargeted metabolomics analyses.[30]

Consolidating Reports

Before they send out data reports to collaborators, GC−MS users overwhelmingly use method blanks, sample blanks, reagent blanks, and quality control pools to curate and consolidate reports (Figure 4). Every respondent reported

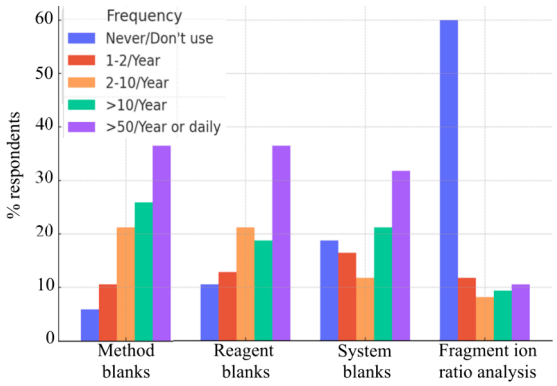

Figure 4. Frequency for how often respondents report using various methods to control for background interferences.

using at least one type of blank in their analyses. More than 90% of the laboratories flagged samples that showed drifts in total intensities (including failed injections) or removed specific peaks that did not pass QC thresholds for missing data (Figure 5). Most of the users also regularly employ other techniques, such as removing peaks that did not pass peak shape QC measures, that failed signal/noise thresholds, or that did not pass QC reproducibility thresholds. Interestingly, almost half of the laboratories did not remove peaks that were detected in samples but not detected in QC pools, likely because such compounds may get diluted down in the sample pooling strategy. For minimum practice, we recommend that practitioners use some form of method to flag peaks and/or correct for drifts in intensity or peak shape across QC samples. Removing peaks is at the discretion of the analyst and depends on the goals of the analysis.

Here, the benefit of GC−MS over high-resolution LC−MS/MS is that nominal mass GC−MS will always record noise ions, enabling users to quantify signal and noise levels even for peaks that are not reliably detected in every sample. Unlike in LC−tandem mass spectrometry, GC−MS electron ionization fragmentations are standardized and robust enough that users typically do not check fragment ion ratios. Analyzing fragment ion ratios across samples can also determine whether the software reliably deconvolves coeluting peaks into distinct mass spectra, particularly in cases where coeluting species share fragment ions. This allows analysts to determine whether they have optimized the processing parameters in their selected software. In contrast, users reported practices that are specific for GC−MS and unnecessary for LC−MS users, for example, removing siloxanes from data sets arising from polysiloxane samplers of volatile compounds, polysiloxane-based column films, or siloxane-based derivatization agents. Background interferences are also manually checked for the appearance of unusual peaks for both total and extracted ion chromato-

https://doi.org/10.1021/acs.analchem.5c06918
Anal. Chem. XXXX, XXX, XXX−XXX

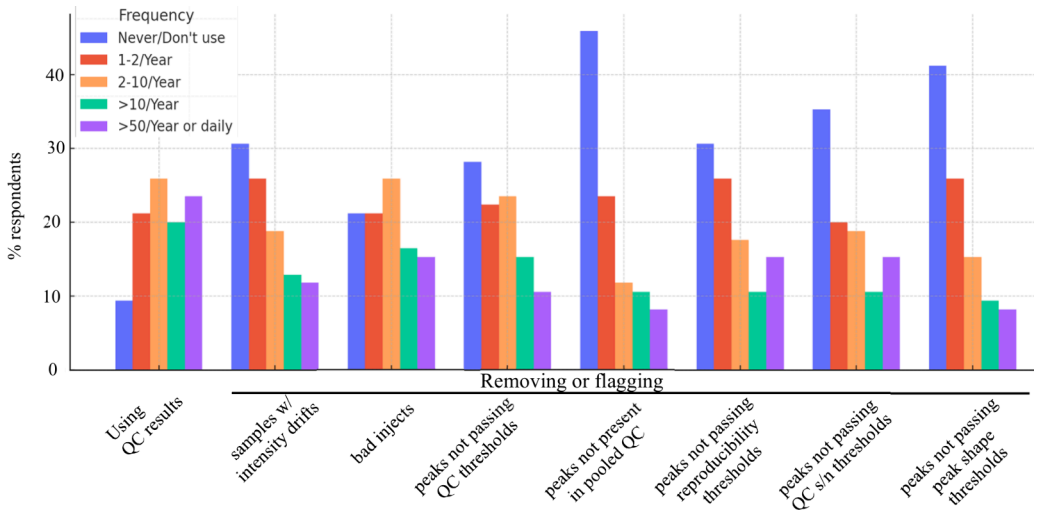

Figure 5. Frequency for how often respondents use various methods to clean and consolidate data sets. QC, quality control; s/n, signal-to-noise.

Table 1. Community-Derived Minimum QA/QC Best Practices for GC−MS and GC × GC−MS-Based Metabolomics

QA/QC practice	recommended minimum best practice	purpose
instrument QA	perform system-specific QA and routine maintenance (e.g., leak checks, tuning, and calibration)	ensures stable instrument performance and data reliability across analytical runs
QC monitoring	inject a standard QC mixture prior to and during analysis; monitor peak shape and intensity and flag deviations[20,21]	enables real-time assessment of instrument performance and early detection of analytical drift
blanks	run blank injections (instrument and reagent blanks) throughout the analysis and report blank data	identifies background contamination and carry-over and prevents false positives; reporting improves data transparency
chromatographic alignment	compute and report retention indices[22,23]	allows for comparability across instruments, methods, and laboratories
internal standards	include appropriate internal standards in all samples	tracks reproducibility and analytical stability across the analysis
chromatographic performance	use peak shape, intensity, and blank response to diagnose changes in instrument response and inform maintenance[20,21]	provides data-driven criteria for instrument troubleshooting and QA/QC
compound annotations	report Metabolomics Standards Initiative (MSI) confidence levels for all identified metabolites[32]	ensures transparency and consistency in metabolite identification and reporting across studies

grams. For forensics use, practitioners reported very strict procedures. Here, any QC check failure may require full reanalysis of any samples that may have been impacted. While all the parameters above are considered, peaks are not removed or flagged, but instead, forensics analysis is suspended until the problem is resolved and the root cause is known and then reperformed in its entirety.

Quantification

In this survey, we specified that targeted metabolomics methods implied the use of calibration standards to report quantitative values. A surprisingly high number of targeted projects are conducted in GC−MS metabolomics laboratories. Eighty-five percent of the users reported at least one project per year using internal standards for quantification, and 36% of the GC−MS metabolomics laboratories use absolute quantifications in their routine practice in >10 projects per year, and only 16% of the respondents never use internal standards for absolute quantifications. In contrast, classic untargeted quantification reports are less frequent. Forty percent of the users reported that they never use sum parameters (such as the sum of internal standards or the sum of all reported peaks) to normalize compound intensities.[31] Likewise, 56% of the GC−MS laboratories never use quality control pool samples to normalize the quantification reports. Pooled samples are less frequently deployed in targeted analyses. It is also important to note that pooling volatile samples can be challenging and sometimes impossible. Given this, analysts should rely on alternative methods, such as internal standards, depending on the sample matrix. Of the three participants reporting analysis of only volatile samples, two reported using pooled samples.

Compound Annotations

Methods such as using external and internal standards, retention index calculations, and mass spectral similarity thresholds support the confidence in compound identification. Most respondents do not report levels of confidence for compound identifications (MSI levels).[32] Only 30% of the participants reported MSI levels in more than 10 projects per year. Instead, compound annotations in the actual data reports typically rely on a combination of retention indices (that have been standardized in GC−MS over 60 years ago) and MS library measures. Analysts using targeted methods may not report MSI levels despite using standards to positively identify metabolites to MSI level 1.[32] Just over half of analysts reporting using exclusively untargeted metabolomics (52%) report MSI levels. Sixty percent of respondents who run at least two GC−MS-based metabolomics projects per year rely on a combination of retention indices and MS library measures. A clear majority of users (72%) regularly employ chemical standards in their projects in more than 2 to >50 projects per year, whereas only a few (8%) respondents stated that they do not use external or internal standards to verify compound annotations. Interestingly, very few projects are conducted that solely rely on retention time matching, and similarly, only a minority of projects rely on annotations that

https://doi.org/10.1021/acs.analchem.5c06918
Anal. Chem. XXXX, XXX, XXX−XXX

exclusively use MS similarity matching. In this way, GC−MS has an advantage for metabolomics, whereas LC−MS/MS retention time standardization (and predictions) is more challenging. One challenge with GC−MS compound annotation is the lack of high-resolution EI spectral libraries, particularly from NIST. As high-resolution and accurate mass GC−MS becomes more common, we expect more commercial libraries will become available.

CONCLUSIONS

Here, we conducted a survey of 85 laboratories from 27 countries on their current practices and use of GC−MS in metabolomics to understand the application, trends, and challenges. Overall, a remarkable consensus of best practices emerged with most laboratories using more than one quality check in reporting. The survey highlighted various QA/QC requirements and considerations in various fields and applications of metabolomics. Table 1 is a summary of the mQACC GC−MS consortium's suggested minimum best practices for QA/QC in GC−MS metabolomics, most of which are broadly adopted by the surveyed community.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.5c06918.

Survey questions and reported country of respondents (PDF)

Results from survey with identifying information removed (XLSX)

AUTHOR INFORMATION

Corresponding Author

Oliver Fiehn − *University of California, Davis, California 95616, United States;* orcid.org/0000-0002-6261-8928; Email: ofiehn@ucdavis.edu

Authors

Ryland T. Giebelhaus − *Department of Chemistry, University of Victoria, Victoria, BC V8P 5C2, Canada;* orcid.org/0000-0002-7625-3077

Michael Herold − *BASF Metabolome Solutions GmbH, Berlin 10589, Germany*

James J. Harynuk − *Department of Chemistry, University of Alberta, Edmonton, AB T6G 2N4, Canada; The Metabolomics Innovation Centre (TMIC), Edmonton, AB T6G 2N4, Canada;* orcid.org/0000-0002-9627-5206

David E. Alonso − *LECO Corporation, St. Joseph, Michigan 49085, United States*

Bashar Amer − *Thermo Fisher Scientific Incorporated, Waltham, Massachusetts 02451, United States*

Corey D. Broeckling − *Analytical Resources Core, Colorado State, Fort Collins, Colorado 80523, United States*

Pantelis Charisiadis − *Precision Exposomics & Health Laboratory, Cyprus International Institute for Environmental and Public Health, School of Health Sciences, Cyprus University of Technology, Limassol 3036, Cyprus;* orcid.org/0000-0001-7260-192X

Raquel Cumeras − *Oncology Department, Institut d'Investigació Sanitària Pere Virgili (IISPV), CERCA, Reus 43204, Spain; Engineering Department, Universitat Rovira i Virgili, Tarragona 43003, Spain;* orcid.org/0000-0003-4663-4247

A. Paulina de la Mata − *Department of Chemistry, University of Alberta, Edmonton, AB T6G 2N4, Canada; The Metabolomics Innovation Centre (TMIC), Edmonton, AB T6G 2N4, Canada; LECO Corporation, St. Joseph, Michigan 49085, United States*

Warwick Dunn − *Department of Biochemistry, Cell and Systems Biology, Centre for Metabolomics Research, Institute of Systems, Molecular, and Integrative Biology, University of Liverpool, Liverpool L69 7ZB, U.K.;* orcid.org/0000-0001-6924-0027

Flavio A. Franchina − *Department of Chemical, Pharmaceutical, and Agricultural Sciences, University of Ferrara, Ferrara 44121, Italy*

Helen Gika − *Department of Medicine, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece;* orcid.org/0000-0002-1893-937X

Jennifer A. Kirwan − *Metabolomics Unit, University of Veterinary Medicine, Wien 1210, Austria; Metabolomics, Berlin Institute of Health at Charité Universitätsmedizin, Berlin 10178, Germany; Metabolomics Platform, Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin 13125, Germany;* orcid.org/0000-0002-5423-1651

Isabella H. Muti − *Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02115, United States*

Lukáš Najdekr − *Faculty of Medicine and Dentistry, Institute of Molecular and Translational Medicine, Palacký University Olomouc, Olomouc 779 00, Czech Republic*

Estelle Pujos-Guillot − *Plateforme d'Exploration du Métabolisme, MetaboHUB Clermont, Université Clermont Auvergne, INRAE, UNH, Clermont-Ferrand 63000, France*

Pierre-Hugues Stefanuto − *Organic and Biological Analytical Chemistry Group, MolSys Research Unit, University of Liège, Liège B4000, Belgium;* orcid.org/0000-0002-1224-8869

Teresa Steininger-Mairinger − *Institute of Analytical Chemistry, BOKU University, Vienna 1190, Austria;* orcid.org/0000-0001-7809-1529

Alpesh Thakker − *Department of Metabolism and Systems Science, School of Medical Sciences, College of Medicine and Health, University of Birmingham, Birmingham B15 2TT, United Kingdom*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.analchem.5c06918

Author Contributions

R.T.G.: preparation of survey, data collection and preparation, writing−review and editing. M.H.: conceptualization, preparation of survey, data analysis, writing−review and editing, administration (cochair of working group). O.F.: conceptualization, preparation of survey, data analysis, writing−review and editing, administration (cochair of working group). H.G.: data analysis, writing−review and editing, and graphical abstract. All other authors contributed equally to developing survey questions, investigation, and manuscript editing. All authors reviewed and approved the manuscript.

Notes

The authors declare no competing financial interest.

https://doi.org/10.1021/acs.analchem.5c06918
Anal. Chem. XXXX, XXX, XXX−XXX

■ ACKNOWLEDGMENTS

This manuscript was prepared on behalf of the mQACC GC–MS working group. We would like to thank all members of mQACC for their support. We would like to extend a sincere thank you to all the participants who took the time to respond to this survey. We would like to acknowledge and thank mQACC members Dr. Dajana Vuckovic (Concordia University) for her close reading, editing, and feedback of this manuscript and Dr. Yunjia Lai (Columbia University Mailman School of Public Health) for her thoughtful comments about the abstract and discussion. C.D.B. is employed in the CSU ARC, Research Resource ID (RRID: SCR_021758). R.C. reports funding from Grant PID2021-126543OB-C22 funded by MICIU/AEI/10.13039/501100011033 and by ERDF/EU.

■ REFERENCES

(1) Blumberg, L. M.Theory of Gas Chromatography. In *Gas Chromatography*; Elsevier, 2021; pp 19–97. .
(2) Fiehn, O. Metabolomics by Gas Chromatography–Mass Spectrometry: Combined Targeted and Untargeted Profiling. *Curr. Protoc. Mol. Biol.* **2016**, *114* (1), 30.4.1–30.4.32.
(3) Higgins Keppler, E. A.; Jenkins, C. L.; Davis, T. J.; Bean, H. D. Advances in the Application of Comprehensive Two-Dimensional Gas Chromatography in Metabolomics. *TrAC Trends Anal. Chem.* **2018**, *109*, 275–286.
(4) Santos, F. J.; Galceran, M. T. The Application of Gas Chromatography to Environmental Analysis. *TrAC Trends Anal. Chem.* **2002**, *21* (9–10), 672–685.
(5) Sun, Q.; Dong, Y.; Wen, X.; Zhang, X.; Hou, S.; Zhao, W.; Yin, D. A Review on Recent Advances in Mass Spectrometry Analysis of Harmful Contaminants in Food. *Front. Nutr.* **2023**, *10*, No. 1244459.
(6) Bajo-Fernández, M.; Souza-Silva, É. A.; Barbas, C.; Rey-Stolle, M. F.; García, A. GC-MS-Based Metabolomics of Volatile Organic Compounds in Exhaled Breath: Applications in Health and Disease. A Review. *Front. Mol. Biosci.* **2024**, *10*, No. 1295955.
(7) Pirok, B. W. J.; Schoenmakers, P. J.*Analytical Separation Science*; Royal Society of Chemistry, 2025. .
(8) Beger, R. D.; Dunn, W. B.; Bandukwala, A.; Bethan, B.; Broadhurst, D.; Clish, C. B.; Dasari, S.; Derr, L.; Evans, A.; Fischer, S.; Flynn, T.; Hartung, T.; Herrington, D.; Higashi, R.; Hsu, P.-C.; Jones, C.; Kachman, M.; Karuso, H.; Kruppa, G.; Lippa, K.; Maruvada, P.; Mosley, J.; Ntai, I.; O'Donovan, C.; Playdon, M.; Raftery, D.; Shaughnessy, D.; Souza, A.; Spaeder, T.; Spalholz, B.; Tayyari, F.; Ubhi, B.; Verma, M.; Walk, T.; Wilson, I.; Witkin, K.; Bearden, D. W.; Zanetti, K. A. Towards Quality Assurance and Quality Control in Untargeted Metabolomics Studies. *Metabolomics* **2019**, *15* (1), 4.
(9) Evans, A. M.; O'Donovan, C.; Playdon, M.; Beecher, C.; Beger, R. D.; Bowden, J. A.; Broadhurst, D.; Clish, C. B.; Dasari, S.; Dunn, W. B.; Griffin, J. L.; Hartung, T.; Hsu, P.-C.; Huan, T.; Jans, J.; Jones, C. M.; Kachman, M.; Kleensang, A.; Lewis, M. R.; Monge, M. E.; Mosley, J. D.; Taylor, E.; Tayyari, F.; Theodoridis, G.; Torta, F.; Ubhi, B. K.; Vuckovic, D. on behalf of the Metabolomics Quality Assurance, Quality Control Consortium (mQACC). Dissemination and Analysis of the Quality Assurance (QA) and Quality Control (QC) Practices of LC–MS Based Untargeted Metabolomics Practitioners. *Metabolomics* **2020**, *16* (10), 113.
(10) Kirwan, J. A.; Gika, H.; Beger, R. D.; Bearden, D.; Dunn, W. B.; Goodacre, R.; Theodoridis, G.; Witting, M.; Yu, L.-R.; Wilson, I. D. the. Quality Assurance and Quality Control Reporting in Untargeted Metabolic Phenotyping: mQACC Recommendations for Analytical Quality Management. *Metabolomics* **2022**, *18* (9), 70.
(11) ISO*International Organization for Standardization* (2021). http://www.iso.org/iso/iso_catalogue/.
(12) Broeckling, C. D.; Beger, R. D.; Cheng, L. L.; Cumeras, R.; Cuthbertson, D. J.; Dasari, S.; Davis, W. C.; Dunn, W. B.; Evans, A. M.; Fernández-Ochoa, A.; Gika, H.; Goodacre, R.; Goodman, K. D.; Gouveia, G. J.; Hsu, P.-C.; Kirwan, J. A.; Kodra, D.; Kuligowski, J.; Lan, R. S.-L.; Monge, M. E.; Moussa, L. W.; Nair, S. G.; Reisdorph, N.; Sherrod, S. D.; Ulmer Holland, C.; Vuckovic, D.; Yu, L.-R.; Zhang, B.; Theodoridis, G.; Mosley, J. D. Current Practices in LC-MS Untargeted Metabolomics: A Scoping Review on the Use of Pooled Quality Control Samples. *Anal. Chem.* **2023**, *95* (51), 18645–18654.
(13) Lippa, K. A.; Aristizabal-Henao, J. J.; Beger, R. D.; Bowden, J. A.; Broeckling, C.; Beecher, C.; Clay Davis, W.; Dunn, W. B.; Flores, R.; Goodacre, R.; Gouveia, G. J.; Harms, A. C.; Hartung, T.; Jones, C. M.; Lewis, M. R.; Ntai, I.; Percy, A. J.; Raftery, D.; Schock, T. B.; Sun, J.; Theodoridis, G.; Tayyari, F.; Torta, F.; Ulmer, C. Z.; Wilson, I.; Ubhi, B. K. Reference Materials for MS-Based Untargeted Metabolomics and Lipidomics: A Review by the Metabolomics Quality Assurance and Quality Control Consortium (mQACC). *Metabolomics* **2022**, *18* (4), 24.
(14) Hussinger, K.; Palladini, L. Information Accessibility and Knowledge Creation: The Impact of Google's Withdrawal from China on Scientific Research. *Ind. Innov.* **2024**, *31* (6), 753–783.
(15) C. Moldoveanu, S.; David, V.;. Derivatization Methods in GC and GC/MS. In *Gas Chromatography - Derivatization, Sample Preparation, Application*; Kusch, P. Ed.; IntechOpen, **2019**. .
(16) Taylor, T.GC Column Killers!*LCGC Int.***2023**194.
(17) Dorman, F. L.; Overton, E. B.; Whiting, J. J.; Cochran, J. W.; Gardea-Torresdey, J. Gas Chromatography. *Anal. Chem.* **2008**, *80* (12), 4487–4497.
(18) Beale, D. J.; Pinu, F. R.; Kouremenos, K. A.; Poojary, M. M.; Narayana, V. K.; Boughton, B. A.; Kanojia, K.; Dayalan, S.; Jones, O. A. H.; Dias, D. A. Review of Recent Developments in GC–MS Approaches to Metabolomics-Based Research. *Metabolomics* **2018**, *14* (11), 152.
(19) Lei, Z.; Huhman, D. V.; Sumner, L. W. Mass Spectrometry Strategies in Metabolomics. *J. Biol. Chem.* **2011**, *286* (29), 25435–25442.
(20) Grob, K.; Grob, G.; Grob, K. Testing Capillary Gas Chromatographic Columns. *J. Chromatogr. A* **1981**, *219* (1), 13–20.
(21) Grob, K.; Grob, G. Practical Capillary Gas Chromatography—a Systematic Approach. *J. High Resolut. Chromatogr.* **1979**, *2* (3), 109–117.
(22) Rostad, C. E.; Pereira, W. E. Kovats and Lee Retention Indices Determined by Gas Chromatography/Mass Spectrometry for Organic Compounds of Environmental Interest. *J. High Resolut. Chromatogr.* **1986**, *9* (6), 328–334.
(23) Curvers, J.; Rijks, J.; Cramers, C.; Knauss, K.; Larson, P. Temperature Programmed Retention Indices: Calculation from Isothermal Data. Part 1: Theory. *J. High Resolut. Chromatogr.* **1985**, *8* (9), 607–610.
(24) Heintz, C.; Kersten, H.; Benter, T.; Wißdorf, W. Signatures of Charged Droplets from ESI: A Statistical Analysis of Non-Summed Mass Spectra Compared to APCI. *J. Am. Soc. Mass Spectrom.* **2025**, *36* (4), 839–849.
(25) Aggio, R. B.; Mayor, A.; Reade, S.; Probert, C. S.; Ruggiero, K. Identifying and Quantifying Metabolites by Scoring Peaks of GC-MS Data. *BMC Bioinformatics* **2014**, *15* (1), 374.
(26) Tsugawa, H.; Cajka, T.; Kind, T.; Ma, Y.; Higgins, B.; Ikeda, K.; Kanazawa, M.; VanderGheynst, J.; Fiehn, O.; Arita, M. MS-DIAL: Data-Independent MS/MS Deconvolution for Comprehensive Metabolome Analysis. *Nat. Methods* **2015**, *12* (6), 523–526.
(27) Cajka, T.; Hajslova, J. Gas Chromatography–Time-of-Flight Mass Spectrometry in Food Analysis. *LCGC Int.* **2007**, *20* (1), 25–31.
(28) Libarondi, M.; Binkley, J. Comparing the Capabilities of Time-of-Flight and Quadrupole Mass Spectrometers. *LCGC Int.* **2010**, *8* (3), 28–33.
(29) Zhu, M.; Zhang, H.; Humphreys, W. G. Drug Metabolite Profiling and Identification by High-Resolution Mass Spectrometry. *J. Biol. Chem.* **2011**, *286* (29), 25419–25425.
(30) Tiller, P. R. Accurate Mass: Why It's the Best Solution for Metabolite Identification in Discovery, Development, and Clinical Applications. *LCGC Int.* **2012**, *10* (1), 12.

https://doi.org/10.1021/acs.analchem.5c06918
Anal. Chem. XXXX, XXX, XXX–XXX

(31) Nam, S. L.; Giebelhaus, R. T.; Tarazona Carrillo, K. S.; de la Mata, A. P.; Harynuk, J. J. Evaluation of Normalization Strategies for GC-Based Metabolomics. *Metabolomics* **2024**, *20* (2), 22.
(32) Sumner, L. W.; Amberg, A.; Barrett, D.; Beale, M. H.; Beger, R.; Daykin, C. A.; Fan, T. W.-M.; Fiehn, O.; Goodacre, R.; Griffin, J. L.; Hankemeier, T.; Hardy, N.; Harnly, J.; Higashi, R.; Kopka, J.; Lane, A. N.; Lindon, J. C.; Marriott, P.; Nicholls, A. W.; Reily, M. D.; Thaden, J. J.; Viant, M. R. Proposed Minimum Reporting Standards for Chemical Analysis. *Metabolomics* **2007**, *3* (3), 211−221.

https://doi.org/10.1021/acs.analchem.5c06918
Anal. Chem. XXXX, XXX, XXX−XXX