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Article

Interlaboratory Comparison of Untargeted Mass Spectrometry Data Uncovers Underlying Causes for Variability

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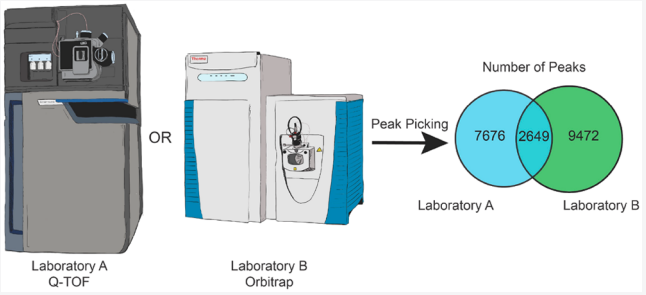
ABSTRACT: Despite the value of mass spectrometry in modern natural products discovery workflows, it remains very difficult to compare data sets between laboratories. In this study we compared mass spectrometry data for the same sample set from two different laboratories (quadrupole time-of-flight and quadrupole-Orbitrap) and evaluated the similarity between these two data sets in terms of both mass spectrometry features and their ability to describe the chemical composition of the sample set. Somewhat surprisingly, the two data sets, collected with appropriate controls and replication, had very low feature overlap (25.7% of Laboratory A features overlapping 21.8% of Laboratory B features). Our data clearly demonstrate that differences in fragmentation, charge state, and adduct formation in the ionization source are a major underlying cause for these differences. Consistent with other recent literature, these findings challenge the conventional wisdom that electrospray ionization mass spectrometry (ESI-MS) yields a simple one-to-one correspondence between analytes in solution and features in the data set. Importantly, despite low overlap in feature lists, principal component analysis (PCA) generated qualitatively similar PCA plots. Overall, our findings demonstrate that comparing untargeted metabolomics data between laboratories is challenging, but that data sets with low feature overlap can yield the same qualitative description of a sample set using PCA.



Mass spectrometry-based metabolomics is emerging as a central tool for determining the chemical composition of natural product samples, including botanical extracts.[1,2] Botanical natural products are often evaluated using targeted methods, where the sample is analyzed to determine the presence of "marker compounds" from a defined target list.[3,4] The inherent disadvantage of this approach is that metabolites outside this target list are not considered. This can result in contaminants being overlooked or lead to incorrect taxonomic or origin assignments. These issues can be addressed using untargeted metabolomics approaches. With untargeted metabolomics, all detectable features (m/z–retention time pairs) are reported in "feature lists", which can subsequently be annotated for known molecules using reference compounds or *in silico* methods. However, there is currently lack of consensus within the natural products community on the optimal methods for acquiring or processing untargeted metabolomics data sets.

Untargeted mass spectrometry workflow design includes a large number of instrumental and analytical parameters, each of which can influence data quality and content. In this study, we analyzed a set of botanical extracts in two independent laboratories on two different mass spectrometry platforms using similar acquisition and processing methods. From these data, we first evaluated the importance of replicate type selection on data quality. We assessed the impact on feature list composition of performing replicate injections in addition to replicate extractions, to provide guidance on replicate type selection. Second, we assessed how closely the results aligned between the two laboratories when acquired under optimized conditions to better understand the opportunities and challenges associated with sharing untargeted metabolomics data sets between research sites.

RESULTS AND DISCUSSION

Botanical Selection. We selected *Camellia sinensis* (green tea) as the test species for this study. *C. sinensis* has been studied extensively for chemical composition.[5−7] Consequently, many of the common constituents are known, and authentic reference samples of both the complex botanical and many of its components are commercially available. These studies were conducted using a set of 37 different sources of green tea, including cut leaves, powders, and supplements (green tea capsules) from 31 commercial vendors (Supporting

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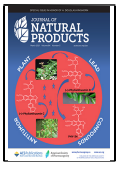

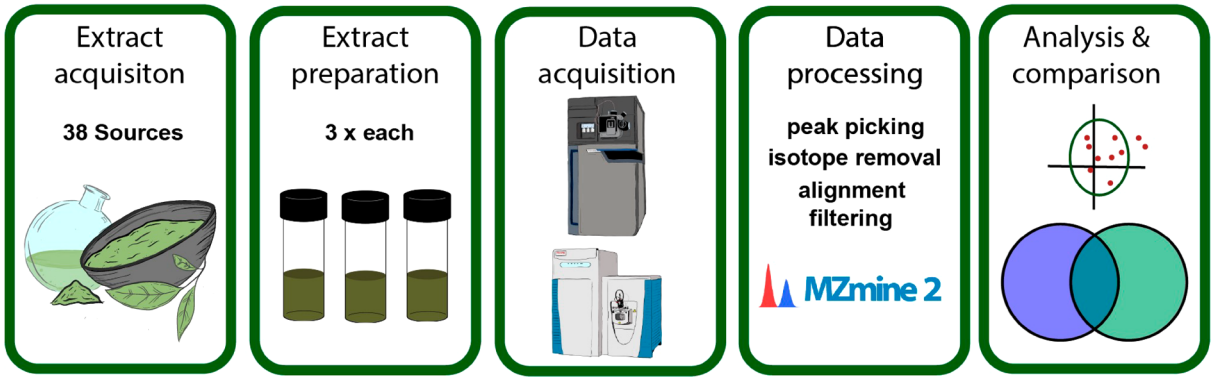

Figure 1. Project workflow.

Information Table S1). These same green tea samples have been used in previous studies in our laboratory, which provided detailed data about their chemical composition.[8] This sample set included the NIST (National Institute of Standards and Technology) green tea leaf standard (SRM 3254), which was used as an authentic green tea (positive) control, and a mixture of *Curcuma longa* and *Zingiber officinale* (turmeric and ginger) tea, which was employed as a non-green tea (negative) control.

Experimental Design. The key considerations for data collection were as follows: the number and type of replicates; instrument type; data acquisition method; chromatographic conditions; and data analysis strategy (Figure 1). To assess the relative influence of performing replicate extractions versus replicate injections, we prepared three separate extractions of each sample and analyzed each extraction replicate by mass spectrometry as injection replicates. Replicate extractions were all prepared by the same researcher using the same source material and equipment. Extracts were concentrated to dryness under nitrogen gas and stored at −20 °C prior to analysis.

For chemical analysis, we employed two different "high-resolution" instruments: a Waters SYNAPT G2-Si qTOF (Laboratory A) and a Thermo Fisher Q-Exactive Plus Orbitrap (Laboratory B). Both instruments were equipped with electrospray ionization (ESI) sources. In addition, the SYNAPT is equipped with a traveling wave ion mobility cell, which was disabled for this study. Both types of instruments (qTOF and Orbitrap) are commonly encountered in academic and industrial natural products research groups and are instruments of choice for many natural product-based metabolomics studies. Although they function in fundamentally different ways, both instruments generate similar types of data (MS^1 and optionally MS^2 data, typically coupled to a liquid chromatography inlet), making it practical to compare the results of analyses. While this study does compare the features detected between two different MS platforms, the goal was not to directly compare the capabilities of these instruments. This is because performance with a single sample type and under a single set of conditions is not representative of the overall performance of any system. For this study the data collected on the Waters SYNAPT G2-Si instrument are labeled Laboratory A, while the data collected in the Thermo Fisher Scientific Q-Exactive Orbitrap are labeled Laboratory B.

Following initial method development (Supporting Information Note S1), a short linear gradient method was selected, and the same column and chromatographic conditions were used on both instruments (Experimental Section). This method balances chromatographic separation against analysis acquisition time. The method was selected as representative of those used for untargeted metabolomics studies of natural products mixtures, rather than being extensively tailored to achieve the best possible separation for *C. sinensis*.

The two mass spectrometers used in the study differ in several important ways with respect to data acquisition parameters. To obtain data sets that were directly comparable, we selected acquisition parameters that could easily be performed on both instruments. Data were obtained in positive ionization mode, using data-dependent acquisition (DDA) for MS^2 data with a maximum of five target masses per MS^1 scan. The same inclusion list of precursor masses (Table S2) was used for all analyses.

As has been clearly demonstrated, data processing software and parameter selection can have a dramatic impact on feature list composition.[9,10] A recent study from Hohrenk et al. showed that processing the same experimental data set using four different software platforms afforded feature lists with a maximum common overlap of 10%.[10] Differences in instrument design and output data formats often complicate data analysis tool selection. For example, the SYNAPT instrument includes a "lockspray" calibrant that is infused into the instrument from a separate nebulizer at a defined interval during the acquisition. These calibration scans complicate processing of the MS^1 data and are not well tolerated by all open-source tools. Similarly, the Orbitrap instrument can be run in fast polarity switching mode, but not all processing packages can correctly identify and segregate these two data types from a single output file. To generate feature lists that were directly comparable between instruments, we selected the popular open-source software platform MZmine 2, which could handle .raw data from both mass spectrometry platforms.[11]

Influence of Replicate Analyses on Data Quality. A number of previous studies have highlighted the importance of performing replicate analyses for untargeted metabolomics analysis of natural products mixtures.[1,12−14] However, analysts often have difficulty determining which type of replication is appropriate. Replication in chemical analysis fits into two broad categories, biological replicates, in which samples are collected from different individuals or pooled samples from a given population (i.e., multiple samples of different plants of the same species or multiple batches of bacterial cells), or analytical replicates, also called technical replicates, where the analysis is repeated multiple times on the same sample.[15] The type of replicate that is appropriate depends entirely on the

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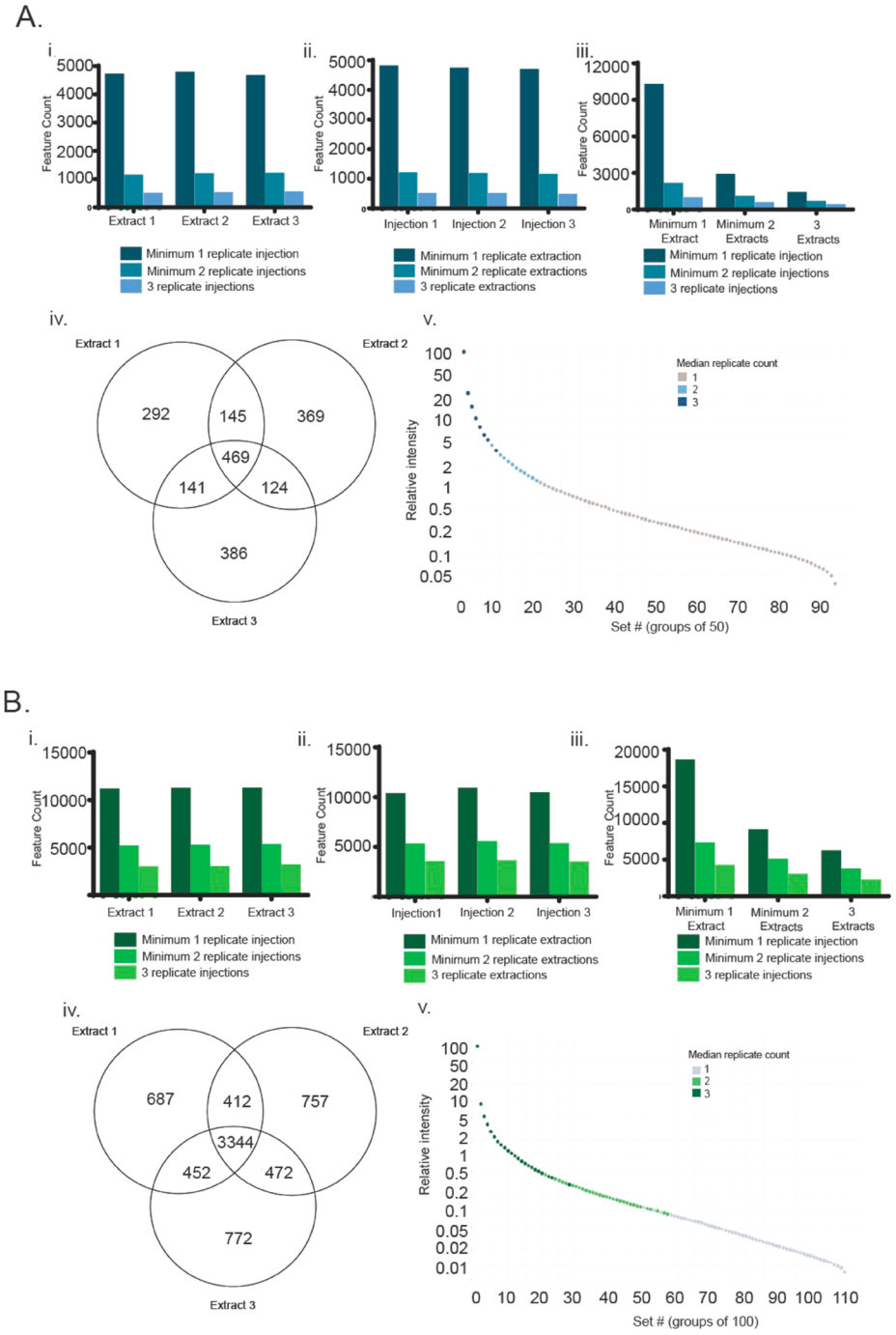

Figure 2. Replicate distributions of features for Laboratories A (blue) and B (green). (i) Distribution of features between replicate injections. (ii) Distribution of features between replicate extractions. (iii) Distribution of features between replicate extractions and replicate injections. (iv) Venn diagram of feature distributions between replicate extractions for features that are present in at least two replicate injections and (v) relationship between feature intensity and replicate count for data from panel (iv).

scientific question being asked. In the test case used for this study, we were not asking a biological question, but rather sought to compare the chemical composition of a series of botanical natural product samples (green tea) all obtained from different suppliers. Thus, we chose to create analytical replicates that captured the variability in the entire process of extraction and analysis of the different samples. We first extracted each sample three times, creating a series of extraction replicates. The question arose in these studies as to whether it would be helpful to conduct replicate analyses of each of these extraction replicates with the mass spectrometer (replicate injections). We addressed this question using the three separate replicate extractions prepared from the same sample of the NIST standard of *C. sinensis* (Experimental Section). Aliquots of each replicate extraction were delivered to Laboratories A and B, resuspended in MeOH, and each extract was analyzed with three replicate injections using the standard column, gradient, mass spectrometer acquisition parameters, and schedule of blank injections (Experimental Section).

Data processing was performed with MZmine 2. The nine replicates of the NIST standard (three replicate injections of

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three replicate extractions) were peak picked using the ADAP workflow (chromatogram builder and deconvolution). The same key ADAP settings were used for data from both instruments (e.g., 5 ppm for feature matching and 0.05 min for retention time tolerance).[16] Intensity filter values were selected individually for each laboratory as the instruments have different sensitivities and dynamic ranges, and samples were therefore injected at different concentrations. Final feature lists were then aligned and filtered (160−1300 m/z and 1−9 min) to remove features from the column wash (9−11 min).

The output from this analysis was a set of nine feature lists from each laboratory, containing the features identified from each replicate analysis. These feature lists were internally consistent in terms of scale, with average feature counts of 2056 ± 49 (mean ± standard deviation) for Laboratory A and 6229 ± 156 for Laboratory B. Using these lists, we examined the variability in feature list composition as a function of replicate count for both replicate extractions and replicate injections (Figure 2). In one case, we grouped data from all three injection replicates for a single extraction replicate (Figure 2 panels Ai and Bi), while in the other case we grouped data from all three extraction replicates for a single injection replicate (Figure 2 panels Aii and Bii). In both cases, we assessed the number of features present in at least one, two, or three replicates. Interestingly, in all cases feature counts were similar, with variation typically < 10%. This was true both within replicate types (i.e., between the three extracts in panel (i)) and between replicate types (i.e., between panels (i) and (ii)). In all cases, a sharp decrease in feature counts was observed between those present in at least one replicate and those present in at least two replicates. On average, 60% of the features in each list were observed only once, highlighting the variability of low-intensity features between replicates.

Interestingly, the absolute numbers of features and the relative changes in feature count were closely aligned between both type of replicate for both laboratories. If variations in extraction protocol had a dramatic impact on the constitution or concentration of individual extracts, then we would have expected to observe a higher rate of decrease in feature counts in replicate extractions versus replicate injections. Instead, we see low variation between the feature counts in the two replicate methods as we increase the requirement to be in at least one, two, or three replicates, suggesting that there is low variation in chemical composition between extraction replicates. Finally, we combined these two replicate methods in panel (iii), which provides the feature counts for all possible combinations of replicate counts. The largest bar (far left) is the count of all features present in at least one injection replicate and one extraction replicate. The smallest bar (far right) provides a count of features present in all three injection replicates of all three extraction replicates (the most stringent criterion). These results illustrate the impact of setting different requirements for feature presence in replicates and the dramatic effect that this can have on feature counts for downstream analysis.

However, feature counts alone are not sufficient to fully assess overlap between analyses. To examine the value of performing injection replicates in addition to extraction replicates, we used the data from panel (iii) to filter the feature lists for each extraction replicate to retain only those features present in at least two injection replicates. Using these filtered lists, we then determined the distribution of features between extraction replicates (Venn diagrams, Figure 2 panels Aiv and Biv). Overall, Laboratory A detected a lower number of features (1926) compared to the feature list from Laboratory B (6896). Furthermore, the distribution of features within each set was quite different, with 46% of features from Laboratory A appearing at least twice and 68% of features appearing at least twice in the data set from Laboratory B. Intrigued by the source of this discrepancy, we ordered the features by intensity and placed them in groups of 50 (Laboratory A) or 100 (Laboratory B) and then plotted median group intensity, color coding the data points by median extraction replicate count (Figure 2 panels Av and Bv). This analysis demonstrates, perhaps unsurprisingly, the relationship between replicate extraction count and intensity. Intense features are consistently present in all three analyses, while weaker features are often present just once or twice in the data set.

Overall, our study shows that a significant portion of the features detectable in a single replicate are not observed in two out of three replicates (Figure 2); therefore, inclusion of injection replicates will significantly reduce feature lists if features appearing only once are removed. The question of the relative value of extraction replicates as compared to injection replicates is best addressed by considering how the data will be interpreted. If the goal of the study is to employ untargeted metabolomics to compare chemical composition between samples (as in the case presented here), rigorous experimental design requires that the replicates reproduce the entire method, from extraction through to analysis by the mass spectrometer. Failure to do so could introduce systematic errors into the data set, whereby variations in extraction efficiency could be interpreted as variability in chemical composition of samples. The importance of conducting replicate extractions for rigorous metabolomics studies appears to be generally accepted by the metabolomics community. For example, in a recent survey, 73% of metabolomics practitioners reported the use of replicate extractions in their analyses.[17] Although there has been no similar survey specifically directed at the natural products community employing metabolomics, it is likely that the use of replicate extractions is not particularly common among natural products scientists. This is at least in part because the goal of many natural products studies is not to compare different sources of the same material, but to profile, as comprehensively as possible, the chemical content of a single natural product extract. For such applications, replicates are advisable to improve data set quality and ensure a robust feature list. However, triplicate injections of a single extract are sufficient if the analyst does not seek to make quantitative comparisons between different extracts. Finally, for the study conducted herein, the data suggest that there is little value to conducing injection replicates of replicate extracts, particularly given the increased instrument time required to analyze these samples (nine analyses per sample rather than three).

Comparison of Feature Lists across Laboratories. An overarching objective of our research programs is the development of methods for the accurate characterization of natural products mixtures using MS-based untargeted metabolomics methods. In contrast to targeted metabolomics, where the goal is to determine the presence of compounds from a fixed target list, untargeted metabolomics aims to generate a complete description of the small molecules (metabolites) present in any mixture. Targeted methods are tolerant of feature lists with very high false positive rates, because these erroneous features can be ignored provided that they do not

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Table 1. (A) Reference Compound Identification from Feature Lists Generated without Isotope Peak Filtering and (B) Reference Compound Identification from Feature Lists Generated with Isotope Peak Filtering in the NIST Leaf Extract (SRM 3254)

A.

Standard	Laboratory A	Both	Laboratory B
Caffeic acid	+		
Caffeine			+
Chlorogenic acid			+
Coumaric acid		-	
Epicatechin		+	
Epicatechin gallate		+	
Epigallocatechin		+	
Gallic acid			+
Gallocatechin		+	
Gallocatechin gallate		+	
Kaempferol		+	
Myricetin		+	
Quercetin		+	
Rutin		+	
Theanine		+	

B.

Standard	Laboratory A	Both	Laboratory B
Caffeic acid		-	
Caffeine			+
Chlorogenic acid		-	
Coumaric acid		-	
Epicatechin		+	
Epicatechin gallate		+	
Epigallocatechin		+	
Gallic acid		-	
Gallocatechin		+	
Gallocatechin gallate		+	
Kaempferol	+		
Myricetin	+		
Quercetin	+		
Rutin		+	
Theanine		+	

accidentally align with the target list. By contrast, untargeted methods aim to create feature lists that contain exclusively features that derive from real molecules in the test mixture. The extent to which this goal is met in a given MS metabolomics study is difficult to evaluate, given the large size of metabolomics data sets and that the samples being analyzed are typically of unknown composition. Here we sought to compare data sets between laboratories to evaluate the extent of overlap as a strategy to address data set quality.

An additional observation from Figure 2 is the discrepancy between the absolute number of features identified in the two laboratories (10 047 from Laboratory A vs 18 247 from Laboratory B). It is tempting to conclude that the data from Laboratory B is in some way "better" because more features were detected. However, if these additional features did not derive from the actual sample, then the results from Laboratory A would be a more accurate representation of the chemical constitution of the test mixture. Alternatively, if the two instruments produce different numbers of adducts and in-source fragments, then the two data sets could be equally valuable for describing composition, even though the absolute numbers of features are very different. We were interested in examining this discrepancy in more depth, to try to understand the source of differences in number of features present in the data sets from the different laboratories.

We asked three questions about the data from these replicated feature lists: (1) How many of a set of known reference compounds were observable in each data set? (2) How many features overlapped between the two data sets? and (3) What was the origin of the unique features from each feature list? Question 1 was designed to determine whether the difference in feature list sizes was due solely to detection limit, and therefore to reduced coverage of low abundance molecules in Laboratory A. Question 2 assessed the issue of coverage from a different perspective, by determining whether the feature list from Laboratory A was a subset of the list from Laboratory B or whether the two lists contained fundamentally different features. Finally, question 3 was designed to evaluate whether we could propose an origin for these unique features, based on differences in background signals or ionization and in-source fragmentation behavior between these two mass spectrometers.

To define a reference set of known chemical constituents of *C. sinensis*, we purchased 15 reference compounds (Table S2) and analyzed them in both laboratories to generate a full suite of UHPLC-MS/MS data on both MS platforms. Manual inspection of the raw MS data for the extract prepared from the NIST standard of *C. sinensis* by Laboratories A and B demonstrated that all 15 reference compounds were detectable, based on retention time and MS feature matching (MS^1 and MS^2 data, Supporting Information Figure S1). The reference compounds covered a broad range of retention times and intensities, from caffeine, which was the strongest signal in the sample, to caffeic acid and gallic acid, both of which were present at intensities close to the intensity cutoff assigned to each instrument.

Feature lists were created from a single injection of each replicate extraction on each instrument, with the requirement that features be present in at least two of three extraction replicates. These feature lists were then compared against the reference table for the commercial reference compounds (Table S2) and presence or absence determined by the detection of the required $[M + H]^+$ adduct within 5 ppm and 0.05 min of the reference table. In addition, we compared the results with (Table 1A) and without (Table 1B) the inclusion of the "isotope peak filtering" option in MZmine 2, which excludes features from the feature list that do not have at least one ^{13}C isotope feature.

Interestingly, the number of identified reference compounds was similar for the feature lists from the two laboratories, notwithstanding the differences in feature counts. Without isotope peak filtering, 10 of the compounds represented in the list of reference compounds were identified by both laboratories, with Laboratory A identifying one additional reference compound and Laboratory B identifying three additional reference compounds. With the inclusion of isotope peak filtering, the number of identified reference compounds decreased, with seven reference compounds identified by both

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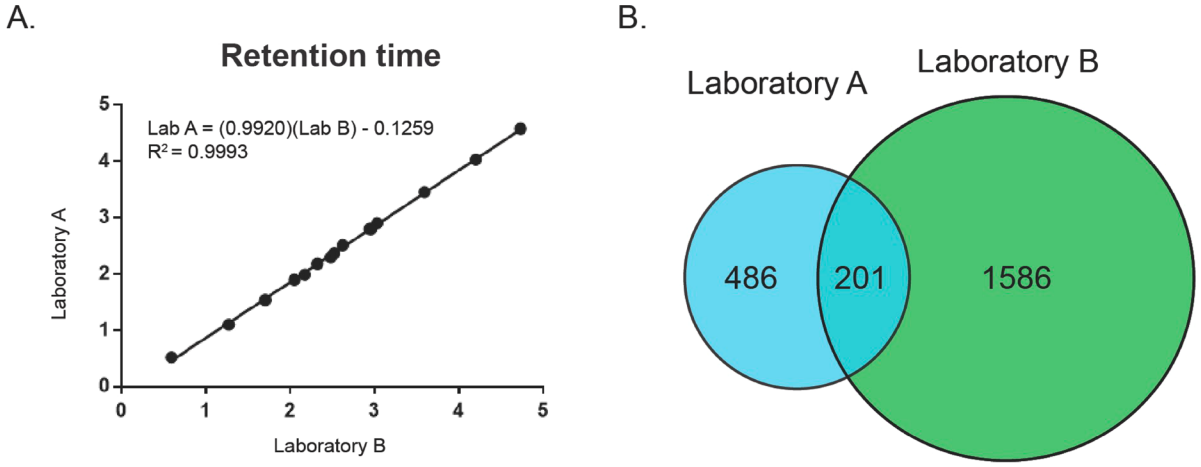

Figure 3. (A) Relationship between retention times for reference compounds detected in the NIST *Camillia sinensis* standard from Laboratory A (*y*-axis) and Laboratory B (*x*-axis). (B) Venn diagram of feature list overlap for NIST standard between Laboratory A (blue) and Laboratory B (green).

laboratories, Laboratory B identifying three additional reference compounds and Laboratory A identifying one additional reference compound. The inclusion of isotope peak filtering selects against low-abundance features because of the requirement to observe at least one additional isotopologue peak, which is typically at an intensity of just a few percent compared to the base all ^{12}C peak for small molecules. Because it negatively impacted the limit of detection for the known reference compounds, the isotope filtering feature was excluded from subsequent analyses.

Manual inspection of the data revealed that gallic acid, chlorogenic acid, and caffeic acid were detected with very low signal intensity in the analysis of the NIST green tea standard extract and were not detected by MZmine 2 in the data set from either laboratory even without the isotope filter. Similarly, coumaric acid was present with such low signal intensity that it was not identified in either data set. By contrast, caffeine was present with a very high signal intensity. This interfered with the mass accuracy in Laboratory A, causing a mass error of >5 ppm and preventing identification of the $[M + H]^+$ signal.

We repeated this analysis on a mixture of all 15 reference compounds at a fixed concentration (6.25 μg/mL Laboratory A; 0.78 μg/mL Laboratory B) using the same chromatographic conditions. Results from this analysis mirrored those from the individual reference compounds, with both laboratories identifying 14 reference compounds, indicating that combining the reference compounds into a single mixture did not impact their detection under these chromatographic conditions.

We next sought to assess what percentage of the features in each list were common between the two data sets. In theory, data acquired on two identical systems should produce two identical feature lists. In reality, however, differences such as background contamination, clustering, and fragmentation within the source, environmental conditions, and column performance will all contribute to differences in feature lists. In this study, differences in feature lists were further increased by the use of two different types of mass spectrometers. A major objective of this study was to assess the degree of similarity between data sets acquired on these two instruments, to evaluate the feasibility of data comparison between the two laboratories.

To relate features in the data sets acquired on the two different instruments, we first considered using a match factor or cosine score between corresponding MS^2 spectra.[18] However, this approach suffers from the major drawback that many of these features are of low intensity and were not selected for DDA MS^2 analysis.

An alternative strategy is to match features between lists based on retention time and mass to charge (m/z) ratio. To be viable as a matching strategy, there must be a defined relationship between the retention times of compounds on the two instruments. Plotting retention time in Laboratory A vs retention time in Laboratory B for the reference compounds demonstrated a strong linear relationship, with an R^2 value of 0.99 (Figure 3A). Using the line of best fit to correct for a small deviation in the void volumes of the two systems (Experimental Section), we compared the corrected retention times and mass to charge ratios between the two feature lists and identified all features that were within 0.1 min retention time and 0.05 Da of one another (Figure 3B).

To our surprise, these feature lists from the two laboratories showed a maximum 29% overlap. Given that both instruments used the same column make and model, elution conditions, and ionization source type (ESI), we expected a higher degree of overlap between feature lists at the MS^1 level. Use of the same column and gradient conditions ensures that the mixture of analytes eluting at a given point in the chromatogram should be similar on each instrument. Under identical ionization conditions, one would expect similar MS^1 signals and, therefore, feature lists of similar magnitude and composition. Several possible justifications for this discrepancy present themselves. First, it is possible that one or both of the feature lists contain a large number of interference features that are not related to true analytes in the mixture. Second, it is possible that different molecules are ionized between the two instruments. Third, it is possible that the two instruments generate significantly different MS^1 features (charge states, fragments, adducts, etc.) for the same set of molecules.

Each of these possibilities raises different concerns for researchers wishing to compare metabolomics data sets between laboratories. Central among these is the question of whether differences in feature lists are due to differing degrees of signal interference, detection of different components of complex mixtures, or different signals from the same set of components. The detection of 11 of 15 reference compounds in both feature lists (Table 1) suggests that in this case

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differences in compound ionization are not a principal driver of feature list variation. All feature lists were subject to blank subtraction to remove chemical interference signals deriving from background chemical contamination (the procedure for blank subtraction is available in the Experimental Section). The requirement that features be present in at least two of three replicates decreases the likelihood that the features outside the combined region of the Venn diagram (Figure 3B) are largely due to electronic interference signals (Figure 1). Instead, we hypothesized that these differences were due in large part to differences in MS^1 signal generation between the two instruments. To test this hypothesis, we analyzed the mixture of the 15 *C. sinensis* reference compounds (Table 1) in triplicate on both instruments. Following the standard processing workflow, we manually identified the $[M + H]^+$ signal for each observable reference compound and, using an in-house Python script that matched features based on alignment of chromatographic peak shapes, assembled all of the MS features associated with each compound. A detailed summary of the data for epicatechin is presented in Figure 4. A comparison of the features for each reference compound from each laboratory is presented in Figure 5.

The results from Figure 5 support the hypothesis that differences between the feature lists from the two instruments are driven to a large extent by differential formation of adducts, clusters, and in-source fragments. Interestingly, although the $[M + H]^+$ signal was present in both data sets in almost all cases, the overall alignment between features was very low, with most compounds having <10% alignment. Also surprising was the observation that very few of the features for each compound could be assigned to common adducts or fragments (black ovals in Venn diagrams above each plot) in either laboratory. This is congruent with several recent reports that have demonstrated that typical untargeted metabolomics feature lists contain vastly more features than compounds and that these features derive at least in part from the generation of many unique features for each analyte during the ionization process.[19−21]

To test the hypothesis that variations in feature list composition between instruments were driven at least in part by differences in MS feature formation for the same set of molecules, we extended our study to a large set of samples containing a higher degree of chemical complexity. If this hypothesis were sound, then independent grouping of related samples based on metabolomic profiles should provide comparable results from both laboratories, regardless of the precise distribution of MS features generated by each analyte. By contrast, if differences in feature list composition were due in large part to chemical interference within the data sets, created by background noise from solvents, atmosphere surrounding the source, or contamination within the liquid chromatograph or mass spectrometer, then either one or both data sets should perform poorly at grouping chemically similar samples.

We prepared three replicate extractions for each of the 37 sources of green tea (Table S1)[8] and the control sample mixture of *C. longa* and *Z. officinale*. This included three NIST standards: NIST 3254 (green tea leaf) and NIST 3255 and 3256 (green tea supplements). We analyzed this sample set independently in Laboratories A and B using the same workflow employed throughout this study and created replicated feature lists for each sample with the requirement that features be present in a minimum of two replicates. A total

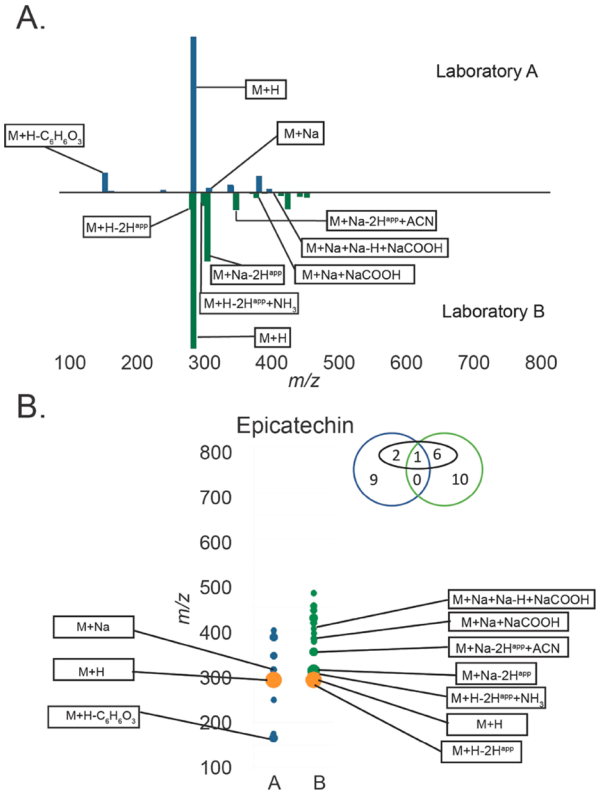

Figure 4. (A) Butterfly plot of MS features for epicatechin in both laboratories and (B) a simplified view of the butterfly plot in panel A. For panel B orange = features present in both data sets, blue = features present only in Laboratory A, green = features present only in Laboratory B. Diameter is proportional to the relative intensity of each data point. The Venn diagram above the trace indicates the number of features from Laboratory A (blue) or Laboratory B (green). Features in black boxes were annotatable adducts or fragments ($[M + H]^+$, $[M + Na]^+$, $[M + H - H_2O]^+$, etc.). Interpretation of the Venn diagram for epicatechin is as follows: 12 and 17 features were grouped with the molecule for Laboratories A and B, respectively. Four of the epicatechin-associated features in the data set for Laboratory A and seven of the epicatechin-associated features in the data set for Laboratory B were annotatable, as adducts or clusters (numbers in black circle). Only one of the epicatechin-associated features was detected by both Laboratory A and Laboratory B, and none (zero) of the unidentified peaks were present in both data sets.

of 10 325 features were detected in the data set obtained by Laboratory A and 12 121 features in the data set from Laboratory B, of which 2649 were detected in both data sets (Figure 6A). These results were broadly aligned with the previous evaluation of the reference compounds' mixture, with ∼20% of the features being present in both data sets.

Next, the two feature lists were subjected to principal component analysis (PCA) to assess their ability to discriminate between sample types, a common and practical application of untargeted metabolomics data sets. Inspection of the PCA scores plot using combinations of the first three components (Figure 6B) yielded similar sample distributions. The non-green tea sample (containing *C. longa* and *Z. officinale*) was located beyond the Hotelling's 95% confidence ellipse in all cases (red dot in Figure 6B), and two green teas that contained additional botanical components (shown in orange in Figure 6B) were spatially located away from the main cluster of green tea samples. The scores plot from the data

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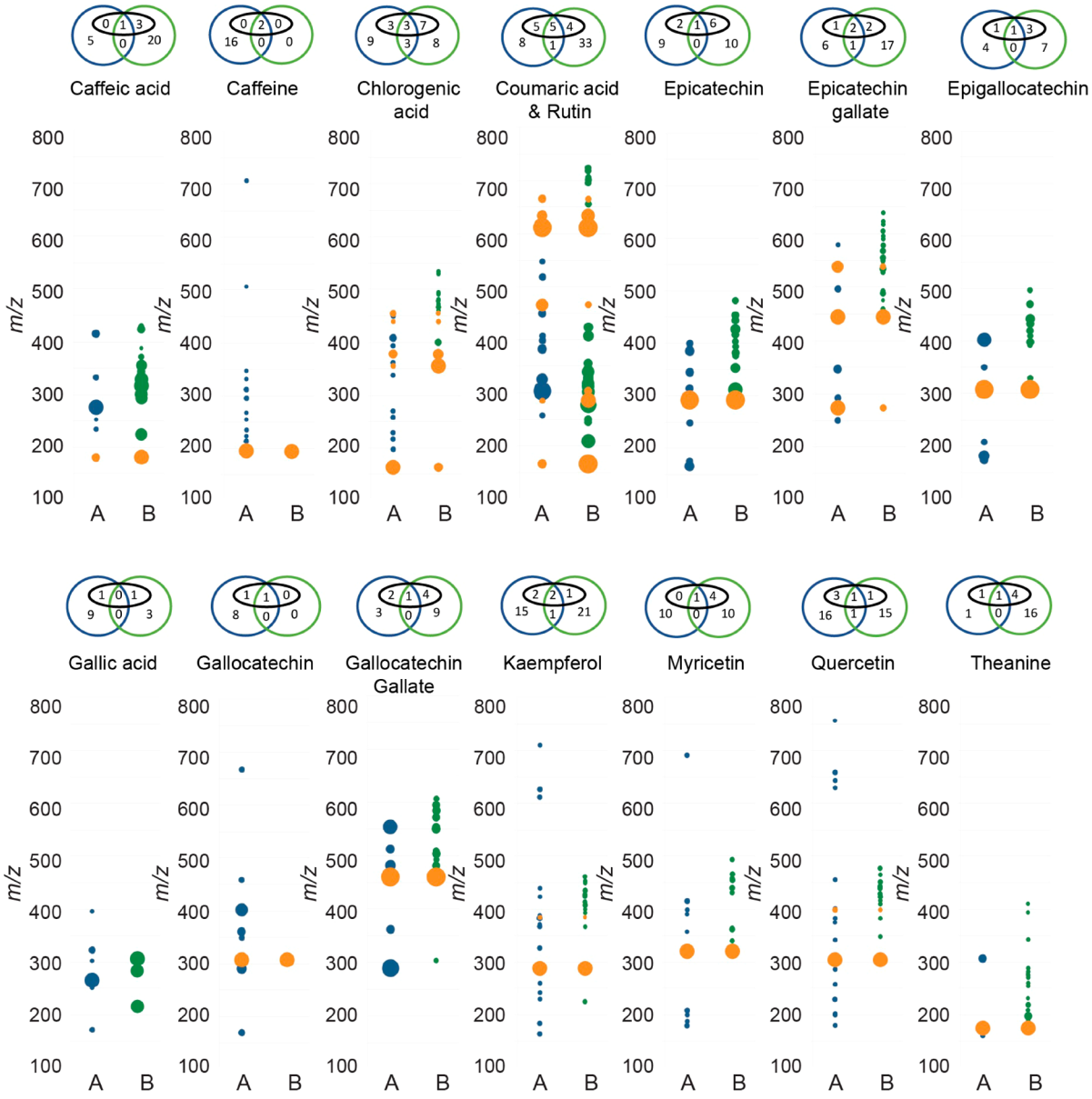

Figure 5. Comparison of MS features for reference compounds analyzed in Laboratories A and B. Orange = features present in both data sets, blue = features present only in Laboratory A, green = features present only in Laboratory B. Diameter proportional to relative intensity. Venn diagram above each trace indicates number of features from Laboratory A (blue) or Laboratory B (green). Features in black ovals were annotatable adducts or fragments ($[M + H]^+$, $[M + Na]^+$, $[M + Na + NaCOOH]^+$, $[M + H - H_2O]^+$, $[2M + Na]^+$, etc.).

from Laboratory A evidenced greater separation between the tea and supplement samples when PC1 versus PC2 was plotted (as compared to the plot of PC2 versus PC3), while the scores plot from Laboratory B showed better discrimination between tea leaves and supplements in the PC2 versus PC3 plot than in the PC1 versus PC2 plot. Overall, the qualitative appearance of the PCA plots, with green tea leaves and supplements grouped separately and NIST standards located within the relevant data clusters, is consistent with the previously published data on this same sample set.[8] The ability to effectively distinguish the various types of samples in the PCA scores plots served to strengthen the conclusion that the differences between feature lists represent "real" features (i.e., those arising from green tea metabolites) and not noise or contamination.

One question that arises when interpreting the data in Figures 5 and 6 is whether the larger number of features observed for Laboratory B compared with Laboratory A could be due to differences in the ability to detect low abundance ions. Under the conditions used in these analyses, the limits of detection for Laboratory A varied depending on the analyte but were typically 5 to 10 times higher than those for Laboratory B (Table 6S). To adjust for this difference, the *C. sinensis* samples were analyzed by Laboratory A at 10-fold higher concentration (1 mg/mL) than for Laboratory B (0.1 mg/mL). Even with the concentrations adjusted to address differences in limit of detection, the feature lists for Laboratory B were still quite different in both identity and number of features as compared to Laboratory A. Thus, we conclude that the differences in feature lists are due at least in part to differences in ionization behavior beyond what could be expected due to differences in limits of detection.

When comparing the differences in data sets across the two laboratories, it is also important to consider the question of whether interpretation might have been confounded by

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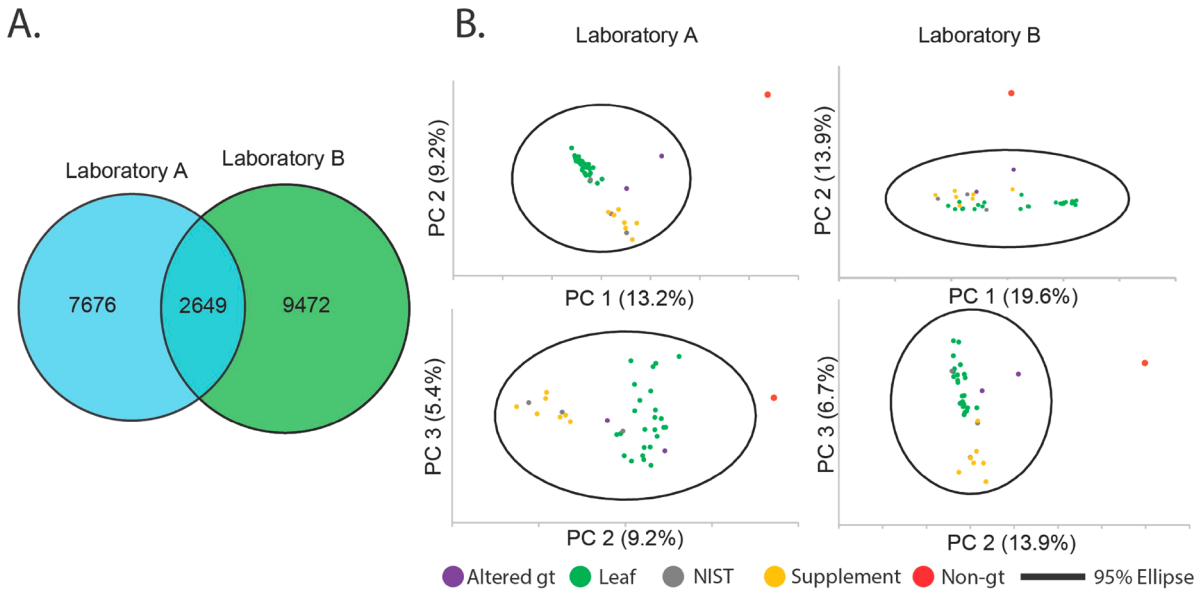

Figure 6. (A) Venn diagram of unique feature list counts and shared features between Laboratories A (blue) and B (green). (B) Principal component analysis (PCA) scores plots of green tea samples: 27 "leaf" products, either whole-leaf teas (21) or powders (6), seven supplements, a single non-green tea ("non-gt", turmeric−ginger tea) negative control, and three *Camellia sinensis* standard reference materials from NIST, drawn with Hotelling's 95% confidence ellipse from Laboratories A (left) and B (right). Top plots are PC1 vs PC2. Bottom plots are PC2 vs PC3.

differences in the acquisition methodologies. For the design of this study, we intentionally attempted to harmonize the data acquisition parameters between the two laboratories to facilitate meaningful comparisons between the data sets acquired on the qTOF (Laboratory A) and the Q-Exactive Orbitrap (Laboratory B). Despite these efforts, there were some minor methodological differences across laboratories that were overlooked in the design step and did not become apparent until the data analysis stage. As described in the Experimental Section, these included the type of solvent used as diluent prior to HPLC injection, the mass spectrometer scan rate, the column temperature, and the number of precursors chosen in the data-dependent analysis. Even with these methodological differences, we contend that the conclusion that different platforms generated different numbers of features and types of adducts detected remains sound. The diluent used prior to injection to LC-MS should have a negligible impact on ionization efficiency (given that it is diluted many-fold in the LC solvent), and the differences in MS scan rate, while impacting duty cycle and sampling across peaks, should not change the identity of the clusters and fragments detected. Any minor differences in retention time that may have arisen from the slightly different column temperatures used were addressed in the process of retention time correction demonstrated in Figure 3.

Importantly, even if all acquisition parameters were identical, it would be impossible to make a head-to-head comparison in the two mass spectrometry platforms due to dramatic differences in the instrument hardware. Although both instruments were equipped with an electrospray ionization source, the source design and ion optics differ between vendors. Furthermore, the two instruments are equipped with different types of mass analyzers, a hybrid quadrupole-time-of-flight for Laboratory A and a hybrid quadrupole-Orbitrap for Laboratory B. Some compromise was necessary to collect data sets with similar parameters on these two instruments, and, consequently, the parameters were not fully optimized for either instrument. Therefore, it is not possible based on this single study to draw a concrete conclusion as to which platform is "better". We can, however, say based on the data presented here that both instruments effectively characterized the chemical differences in the *C. sinensis* samples.

CONCLUSION

The majority of previous interlaboratory comparisons in MS have focused on targeted analysis: comparison of the abundance of known analytes in the data sets from different laboratories.[22−24] This study attempted an interlaboratory comparison using an untargeted approach, comparing feature lists generated independently by each laboratory on the same sample set. We showed that retention times were strongly correlated between the data sets and could be used to facilitate feature-by-feature comparisons. In comparing the data sets, we observed dramatic differences in feature identity, which appear to result from differences in the adducts, fragments, charge states, and clusters generated by different mass spectrometers. Importantly, data sets with very different feature composition collected in two independent laboratories still effectively described qualitative differences in samples, which is typically the stated objective of metabolomics studies.

Our studies have some interesting implications for the community of scientists seeking to employ untargeted metabolomics to characterize natural products samples. First, we demonstrated that the requirement that a feature be detected in at least two of three replicates results in a dramatic reduction in the number of features in an untargeted metabolomics data set. Thus, the inclusion of replicates is important for improving data set quality and preventing erroneous data interpretation, but will also result in rejection of some low-abundance features that may represent real analytes. Second, these data suggest that unique feature lists are to be expected when analyses are conducted on different instruments, an observation that makes the typical interlaboratory comparisons for validating analytical approaches very difficult.

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Our findings emphasize the important conclusion that untargeted metabolomics feature lists are not a description of the chemical constitution of the sample, but rather an instrument-specific snapshot of how the chemical entities in the sample respond to analysis by the particular mass spectrometer. As such, feature lists are useful for comparing samples all analyzed on the same MS platform, but such comparisons should not be extended to samples analyzed on different platforms or in different laboratories. Results from the evaluation of standards in both laboratories highlight the large number of adducts, fragments, and charge states that can derive from a single compound. As has previously been noted, these inflate feature list counts and artificially increase the assumed sample complexity in many cases. An effective and accurate deconvolution tool to reduce a complex feature list down to its component list of individual analytes (chemical entities) would greatly improve the ability to compare MS data sets across laboratories.

EXPERIMENTAL SECTION

Chemicals. Unless otherwise noted, all chemicals were of reagent or spectroscopic grade and obtained from Fisher Scientific

Green Tea Product Selection and Extraction. Commercially available green tea products were selected using consumer sales[25] and product quality reports.[26,27] The 34 products included 21 whole-leaf teas, six powders, and seven supplements (Table S1). A single non-green tea (turmeric−ginger tea) was included as a negative control (GT23), and *Camellia sinensis* standard reference materials from NIST for loose leaf tea (GT26), supplement (GT27), and oral dosage form (GT37) (nos. 3254, 3255, and 3256, respectively) served as positive controls (Table S1). Two green teas that contained other botanical additives were also selected for comparison (GT24 and GT38). A retention sample of each product, containing several grams of material, was maintained in Laboratory B for future reference. Data about the chemical composition of the green tea samples used in this study are publicly available through the Center of Excellence for Natural Product Drug Interaction Research Data Repository, https://repo.napdi.org/.

Green tea products were extracted in triplicate. For each sample, to 200 mg of tea sample was added 20 mL of reagent-grade MeOH, and the mixtures were shaken overnight at room temperature, filtered, concentrated to dryness under nitrogen gas, and stored at −20 °C prior to analysis.

Sample Preparation. Weighed aliquots of dried samples prepared in Laboratory B (~10 mg each) were transferred to new 1-dram vials and shipped to Laboratory A over dry ice. Laboratory A prepared the sample in Optima grade MeOH to a concentration of 5 mg/mL. Vials were then sonicated and vortexed before being diluted to a final concentration of 1 mg/mL in a mixture of 50:50 MeOH−H_2O. Laboratory B reconstituted the dried samples to 4 or 6 mg/mL in Optima grade MeOH depending on the volume limits of the vial. Vials were then sonicated and vortexed before being diluted to a final concentration of 0.1 mg/mL with Optima grade MeOH.

A set of mixes was also created using 15 reference compounds purchased from ChromaDex. For consistency, the mixtures were prepared in Laboratory B and divided into two, and one aliquot was shipped to Laboratory A over dry ice. Mixtures were made in equivalent amounts of each reference compound beginning with 100 μg/mL and following half dilutions ending with 0.195 μg/mL.

LCMS Conditions. A total of nine samples (3 replicate extractions × 3 replicate injections) were injected for green tea sample 26 (GT-26; NIST sample SRM 3254). All other green tea samples were collected as single injections of replicate extractions. Additionally, each of the reference compound mixes were injected in triplicate. Approximately 20% of the sample lists were blank samples dispersed throughout, and three were selected from each lab to be used for blank subtraction.

All measurements were performed with an Acquity I-Class UPLC (Waters) for Laboratory A or Acquity H-class UPLC (Waters) for Laboratory B, with both laboratories using an HSS T3 C_{18} column (100 mm × 2.1 mm, 1.8 μm, Waters). Separation of 5 μL of sample was achieved by a gradient of (A) H_2O + 0.1% formic acid (FA) to (B) MeCN + 0.1% FA at a flow rate of 500 μL/min and for 12.8 min (5% MeCN, 0−0.3 min; 5−90% MeCN, 0.3−9.1 min; 90−98% MeCN, 9.1−10.7 min; 98% MeCN, 10.7−11 min; 5% MeCN, 11.01−12.8 min).

For Laboratory A, the LC flow was directly infused into a SYNAPT G2-Si qTOF (Waters) operated in positive ion mode. Analysis was conducted using DDA mode with an inclusion list for the reference compounds and a maximum of three MS^2 acquisitions per MS^1 survey scan. The instrument was operated in electrospray mode with 200 pg/mL leucine enkephalin lockspray infusion enabled every 10 s. Mass spectra were acquired from 50 to 1500 m/z at a 5 Hz scan rate in centroid mode with lockmass correction.

For Laboratory B the LC flow was directly infused into a Q-Exactive Plus quadrupole-Orbitrap mass spectrometer (Thermo Scientific) operated in positive ion mode. Analysis was conducted using DDA mode with an inclusion list for the reference compounds and a maximum of five MS^2 acquisitions per MS^1 survey scan. Mass spectra were acquired from 120 to 1200 m/z at a 11 Hz scan rate in profile mode.

Data Analysis. All samples were processed using MZmine 2 (http://mzmine.github.io/) version 2.51.[11] Peak detection was performed using mass detection and the ADAP chromatogram builder module using MS level 1, an m/z tolerance of 0.001 Da or 5 ppm, and a minimum group size of four scans for both laboratories. For peak detection Laboratory A used a mass detection (noise level) and group intensity threshold of zero and a minimum highest intensity of 600; Laboratory B used a mass detection (noise level) of 1×10^4, a group intensity threshold of 5×10^4, and a minimum highest intensity of 1×10^5. Deconvolution was then performed using ADAP deconvolution with a signal-to-noise threshold of 10, peak duration range of 0−3, and a retention time wavelet range of 0−0.1 for both laboratories. Laboratory A used a coefficient area threshold of 50 and a minimum feature height of 600, while Laboratory B used a coefficient area threshold of 120 and a minimum feature height of 1×10^5. Feature lists were then deisotoped using an m/z tolerance of 0.001 Da or 5 ppm and a retention time tolerance of 0.05 min. Feature lists for each sample (in triplicate) were then join aligned using a weight of 10 for both m/z and retention time and a retention time tolerance of 0.05 min. The join aligned list was then row filtered for the mass range 160−1300 m/z and time range of 0−9 min and requiring occurrence in a minimum of two out of three samples. The new aligned and filtered lists for each sample were then join aligned together along with a list of blank samples processed identically to the samples, followed by deletion of features that showed up at least two of three times in the blank samples.

Comparison of Feature Lists. Feature lists were compared using an in-house Python script (https://github.com/liningtonlab/green_tea_ms). The script first performs a correction to the retention times for each feature from laboratory A using the line of best fit from Figure 3A and the following equation:

$$\text{corrected retention time} = (\text{retention time} - \text{intercept})/\text{slope}$$

Second, the script identifies features between the two lists that have matching m/z and corrected retention times within a 0.05 Da (m/z window) and 0.1 min (retention time window).

Feature Grouping and Annotation of Reference Compound Mixes. To group features from each feature list, first the scan-by-scan data for each feature was exported from MZmine 2.0 as a csv file. An in-house Python script (https://github.com/liningtonlab/green_tea_ms) was then used on the scan-by-scan data to compare intensities of pairs of features as a function of scan number. Plotting intensity versus intensity as a function of scan number provides a measure of the change in relative intensity for each feature pair. Features that displayed an $r^2 > 0.9$ from the linear regression of these plots were grouped together and defined as a single analyte. Features associated

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with each analyte were retained if they were associated with that analyte in at least two out of three replicate injections.

In MZmine 2, MS^1 annotations were performed through the identification modules (custom database and adduct searches). In addition to the calculated $[M + H]^+$ of each reference compound, the platform MZedDB was employed to generate lists of potential adducts specific to each reference compound.[28] For additional adducts and neutral losses, a list was compiled using reference data from several different sources.[29−34] The annotation modules were employed with an m/z tolerance of 0.002 Da or 5 ppm and a retention time tolerance of 0.05 min. A maximum relative adduct peak height of 10 000% was set for adducts and neutral masses. The annotation results are reported as $[M + CC + NM]^+$ according to the model proposed by Kachman et al.,[31] where CC denotes the charge carrier and NM is the proposed neutral mass (gain or loss). The lists compiling the adducts and neutral losses are presented in Table S5.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c01376.

Additional methods (Note S1), green tea sample sourcing details (Table S1), green tea reference compound identity and details (Table S2), presence of reference compounds in green tea samples (Table S3), MS^2 butterfly plots for each standard (Figure S1), annotation details for both Laboratory A (Table S4A) and Laboratory B (Table S4B), adduct/fragment distribution per standard analyte (Figure S2), detailed source material for possible adduct and fragment annotation (Table S5a−c), blank injection chromatograms (Figure S3), EIC chromatograms of the reference compounds (Figure S4), and LOD comparison between the two instruments (Table S6) (PDF)

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

Dedicated to Dr. A. Douglas Kinghorn, The Ohio State University, for his pioneering work on bioactive natural products.

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SUPPORTING INFORMATION

Interlaboratory Comparison of Untargeted Mass Spectrometry Data Uncovers Underlying Causes for Variability

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CONTENTS

Note S1.

Gradient selection was designed to identify conditions that balanced separation of the standard compounds (Supplementary Table S2) against overall acquisition time. Three gradients were chosen for testing:

1) A gradient of (A) H2O + 0.1% FA to (B) ACN + 0.1% FA at a flow rate of 500 µL/min and 45°C for 7.6 min (5% ACN, 0-0.3 min; 5-90% ACN, 0.3-4.7 min; 90-98% ACN, 4.7-5.5 min; 98% ACN, 5.5-5.8 min; 5% ACN, 5.81-7.6 min).

2) A gradient of (A) H2O + 0.1% FA to (B) ACN + 0.1% FA at a flow rate of 500 µL/min and 45°C for 12.8 min (5% ACN, 0-0.3 min; 5-90% ACN, 0.3-9.1 min; 90-98% ACN, 9.1-10.7 min; 98% ACN, 10.7-11 min; 5% ACN, 11.01-12.8 min).

3) A gradient of (A) H2O + 0.1% FA to (B) ACN + 0.1% FA at a flow rate of 500 µL/min and 45°C for 17.8 min (5% ACN, 0-0.3 min; 5-90% ACN, 0.3-14 min; 90-98% ACN, 14-15.6 min; 98% ACN, 15.6-15.9 min; 5% ACN, 15.91-17.8 min).

Gradient 2 was the shortest gradient that achieved separation of the standards; gradient 2 was therefore selected for this study.

Table S1. Selection and coding of commercial green tea products under investigation

code	product form	selection criteria
GT 1	leaf tea	consumer sales[1]
GT 2	leaf tea	quality report[2]
GT 3	leaf tea	consumer sales[1]
GT 4	leaf tea	quality report[2]
GT 5	leaf tea	quality report[2]
GT 6	leaf tea	quality report[2]
GT 7	leaf tea	quality report[2]
GT 8	leaf tea	quality report[2]
GT 9	leaf tea	quality report[2]
GT 10	leaf tea	quality report[2]
GT 11	powdered tea	consumer sales[1]
GT 12	powdered tea	quality report[2]
GT 13	leaf tea	quality report[2]
GT 14	leaf tea	quality report[2]
GT 15	tea supplement	quality report[2]
GT 16	leaf tea	quality report[2]
GT 17	leaf tea	quality report[2]
GT 18	leaf tea	quality report[2]
GT 19	tea supplement	quality report[2]
GT 20	leaf tea	quality report[2]
GT 21	leaf tea	quality report[2]
GT 22	powdered tea	quality report[2]
GT 23	leaf tea	non-green tea
GT 24	leaf tea	consumer sales[1]
GT 25	tea supplement	extract report[3]
GT 26	leaf tea	NIST standard
GT 27	tea supplement	NIST standard
GT 28	tea supplement	extract report[3]
GT 29	tea supplement	extract report[3]
GT 30	powdered tea	consumer sales[1]
GT 31	powdered tea	consumer sales[1]
GT 32	powdered tea	consumer sales[1]
GT 33	leaf tea	quality report[2]
GT 34	leaf tea	quality report[2]
GT 35	tea supplement	extract report[3]
GT 36	tea supplement	extract report[3]
GT 37	tea supplement	NIST standard
GT 38	leaf tea	consumer sales[1]

Table S2. Green tea reference compounds used as quality control checks for green tea samples from Table S1

	reference compounds	formula	$[M+H]^+$	RT* in lab A	RT in lab B
1	caffeic acid	$C_9H_8O_4$	181.0501	2.30	2.48
2	caffeine	$C_8H_{10}N_4O_2$	195.0883	2.18	2.32
3	chlorogenic acid	$C_{16}H_{18}O_9$	355.1029	1.99	2.17
4	coumaric acid	$C_9H_8O_3$	165.0552	2.80	2.96
5	(-)-epicatechin	$C_{15}H_{14}O_6$	291.0869	2.37	2.52
6	(-)-epicatechin gallate	$C_{22}H_{18}O_{10}$	443.0978	2.90	3.03
7	(-)-epigallocatechin	$C_{15}H_{14}O_7$	307.0818	1.90	2.05
8	gallic acid	$C_7H_6O_5$	171.0294	1.10	1.27
9	(-)-gallocatechin	$C_{15}H_{14}O_7$	307.0818	1.54	1.70
10	(-)-gallocatechin gallate	$C_{22}H_{18}O_{11}$	459.0927	2.51	2.62
11	kaempferol	$C_{15}H_{10}O_6$	287.0556	4.58	4.73
12	myricetin	$C_{15}H_{10}O_8$	319.0454	3.45	3.59
13	quercetin	$C_{15}H_{10}O_7$	303.0505	4.03	4.20
14	rutin	$C_{27}H_{30}O_{16}$	611.1612	2.80	2.94
15	theanine	$C_7H_{14}N_2O_3$	175.1083	0.52	0.59

*RT = retention time (min)

Table S3. Presence of reference compounds detected in Green tea samples

		Standards															
		caffeic acid	caffeine	chlorogenic acid	coumaric acid	epicatechin	catechin	epicatechin gallate	epigallocatechin	gallic acid	gallocatechin	gallocatechin gallate	kaempferol	myricetin	quercetin	rutin	theanine
Lab A	GT 1 R1	+	+	-	-	+	-	+	+	-	+	+	+	-	-	+	+
	GT 1 R2	+	+	-	-	+	+	+	+	-	+	+	+	+	-	+	+
	GT 1 R3	+	+	-	-	+	+	+	+	-	+	+	+	+	-	-	+
	GT 2 R1	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 2 R2	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 2 R3	+	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+
	GT 3 R1	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 3 R2	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 3 R3	-	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 4 R1	+	+	-	-	+	-	+	+	-	+	+	+	+	+	+	+
	GT 4 R2	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 4 R3	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 5 R1	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 5 R2	+	+	-	-	+	+	+	+	-	+	+	-	+	+	+	+
	GT 5 R3	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	+
	GT 6 R1	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
	GT 6 R2	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
	GT 6 R3	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
	GT 7 R1	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 7 R2	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
	GT 7 R3	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
	GT 8 R1	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 8 R2	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 8 R3	+	+	-	-	+	+	+	+	-	+	+	+	-	+	+	+
	GT 9 R1	-	-	-	-	+	+	+	+	-	+	+	+	+	-	-	+
	GT 9 R2	-	+	-	-	+	+	+	+	-	+	+	+	+	-	+	+
	GT 9 R3	-	+	-	-	+	+	+	+	-	+	+	+	+	-	+	+
	GT 10 R1	+	-	-	-	+	+	+	+	+	+	+	+	-	-	+	+
	GT 10 R2	+	+	-	-	+	-	+	+	+	+	+	+	-	-	+	+
	GT 10 R3	+	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+
	GT 11 R1	+	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+
	GT 11 R2	+	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+
	GT 11 R3	+	-	-	-	+	+	+	+	-	+	+	-	-	-	+	+
	GT 12 R1	+	+	-	-	+	+	+	+	-	+	+	+	+	-	+	+
	GT 12 R2	+	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+
	GT 12 R3	-	+	-	-	+	-	+	+	-	+	+	+	+	-	+	+
	GT 13 R1	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 13 R2	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 13 R3	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 14 R1	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 14 R2	+	+	-	-	+	+	+	+	-	+	+	+	-	+	+	+
	GT 14 R3	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 15 R1	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-
	GT 15 R2	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-
	GT 15 R3	+	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-
	GT 16 R1	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 16 R2	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 16 R3	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 17 R1	+	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+
	GT 17 R2	+	-	-	-	-	+	+	+	-	+	+	+	+	+	-	+
	GT 17 R3	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 18 R1	-	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 18 R2	+	-	-	-	+	+	+	+	-	-	+	+	+	+	+	+
	GT 18 R3	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+
	GT 19 R1	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-
	GT 19 R2	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+
	GT 19 R3	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+
	GT 20 R1	+	+	-	-	+	+	+	+	-	+	+	+	+	-	+	+
	GT 20 R2	+	+	-	-	+	+	+	+	-	+	+	+	+	-	-	+
	GT 20 R3	-	+	-	-	+	+	+	+	-	+	+	+	+	-	+	+
	GT 21 R1	+	+	-	-	+	+	+	+	-	+	+	+	-	+	-	+
	GT 21 R2	-	-	-	-	+	+	+	+	-	+	+	+	+	+	-	+
	GT 21 R3	+	+	-	-	+	-	+	+	-	+	+	+	+	+	-	+
	GT 22 R1	+	+	-	-	+	+	+	+	-	+	+	+	-	-	-	+
	GT 22 R2	+	+	-	-	+	+	+	+	-	+	+	-	+	-	+	+
	GT 22 R3	+	+	-	-	+	+	+	+	-	+	+	+	+	-	+	+
	GT 23 R1	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	GT 23 R2	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	GT 23 R3	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	GT 24 R1	+	-	-	-	+	+	+	+	-	+	+	-	-	-	+	-
	GT 24 R2	+	-	-	-	+	+	+	+	-	+	+	+	-	+	+	+
	GT 24 R3	+	-	-	-	+	+	+	+	-	+	+	+	-	+	+	+
	GT 25 R1	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+

	GT 25 R2	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
	GT 25 R3	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
	GT 26 R1	+	-	-	-	+	-	+	+	-	+	+	+	+	+	-	+
	GT 26 R2	+	+	-	-	+	+	+	+	-	+	+	+	+	-	-	+
	GT 26 R3	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+
	GT 27 R1	+	+	-	-	+	+	+	+	+	+	-	+	+	+	-	+
	GT 27 R2	-	-	-	+	+	+	+	+	+	+	-	+	+	+	-	-
	GT 27 R3	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	+
	GT 28 R1	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+
	GT 28 R2	+	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+
	GT 28 R3	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+
	GT 29 R1	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-
	GT 29 R2	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-
	GT 29 R3	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-
	GT 30 R1	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	+
	GT 30 R2	+	+	-	-	-	-	+	+	+	+	+	+	-	-	+	+
	GT 30 R3	+	+	-	-	-	-	+	+	+	+	+	+	-	-	+	+
	GT 31 R1	+	+	-	-	-	-	+	+	-	+	+	-	-	-	-	+
	GT 31 R2	-	+	-	-	+	+	+	+	-	+	+	+	-	-	-	+
	GT 31 R3	+	+	-	-	+	+	+	+	-	+	+	+	-	-	-	+
	GT 32 R1	+	+	-	-	+	+	+	+	-	+	+	-	+	-	-	+
	GT 32 R2	+	-	-	-	+	+	+	+	-	+	+	+	+	-	-	+
	GT 32 R3	+	+	-	-	+	+	+	+	-	+	+	+	+	-	-	+
	GT 33 R1	+	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+
	GT 33 R2	+	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+
	GT 33 R3	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 34 R1	+	+	-	-	+	+	+	+	-	+	+	+	-	-	+	+
	GT 34 R2	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 34 R3	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 35 R1	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	-
	GT 35 R2	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-
	GT 35 R3	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-
	GT 36 R1	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 36 R2	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-
	GT 36 R3	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
	GT 37 R1	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+
	GT 37 R2	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	+
	GT 37 R3	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+
	GT 38 R1	-	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+
	GT 38 R2	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+
	GT 38 R3	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+
Lab B	GT 1 R1	+	+	+	-	+	+	-	+	-	-	+	+	+	+	+	+
	GT 1 R2	+	+	+	-	+	+	-	+	-	-	+	-	+	+	+	+
	GT 1 R3	+	+	+	-	+	+	-	+	-	-	+	+	+	+	+	+
	GT 2 R1	+	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+
	GT 2 R2	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
	GT 2 R3	-	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
	GT 3 R1	+	+	+	-	+	+	-	+	-	+	+	+	-	+	+	+
	GT 3 R2	+	-	+	-	+	+	-	+	-	-	+	+	-	+	+	+
	GT 3 R3	+	+	+	-	+	+	-	+	-	+	+	-	-	+	+	+
	GT 4 R1	-	-	+	-	+	+	-	+	-	+	+	+	-	+	+	+
	GT 4 R2	-	-	+	-	+	+	-	+	-	+	+	+	-	+	+	+
	GT 4 R3	-	-	+	-	+	+	-	+	-	+	+	+	-	+	+	+
	GT 5 R1	+	-	+	-	+	+	-	+	-	+	+	+	-	+	+	+
	GT 5 R2	+	-	+	-	+	+	-	+	-	+	+	+	-	+	+	+
	GT 5 R3	+	-	+	-	+	+	-	+	-	+	+	+	-	+	+	+
	GT 6 R1	-	+	+	-	+	+	-	+	-	+	+	+	-	+	+	+
	GT 6 R2	-	+	+	-	+	+	+	+	-	+	+	+	-	+	+	+
	GT 6 R3	-	+	+	-	+	+	+	+	-	+	+	-	-	+	+	+
	GT 7 R1	+	+	+	-	+	+	+	+	-	+	+	+	-	+	+	+
	GT 7 R2	+	+	+	-	+	+	+	+	-	+	+	+	-	+	+	+
	GT 7 R3	+	+	+	-	+	+	+	+	-	+	+	-	-	+	+	+
	GT 8 R1	+	+	+	-	+	+	+	+	-	+	+	-	+	+	+	+
	GT 8 R2	+	+	+	-	+	+	+	+	-	+	+	-	+	+	+	+
	GT 8 R3	+	+	+	-	+	+	+	+	-	+	+	-	-	+	+	+
	GT 9 R1	+	+	+	-	+	+	+	+	-	-	+	-	-	-	+	+
	GT 9 R2	+	+	+	-	+	+	+	+	-	-	+	-	-	-	+	+
	GT 9 R3	+	+	+	-	+	+	+	+	-	-	+	-	-	-	+	+
	GT 10 R1	+	+	+	-	+	+	+	+	-	-	+	-	-	-	+	+
	GT 10 R2	+	+	+	-	-	+	+	+	-	-	+	-	-	-	+	+
	GT 10 R3	+	+	+	-	+	+	+	+	-	-	+	-	-	-	+	+
	GT 11 R1	+	+	+	-	+	+	+	+	-	-	+	-	-	-	+	+
	GT 11 R2	+	+	+	-	+	+	+	+	-	-	+	-	-	-	+	+
	GT 11 R3	+	+	+	-	+	+	+	+	-	-	+	-	-	-	+	+
	GT 12 R1	+	+	+	-	+	+	+	+	-	-	+	-	-	+	+	+
	GT 12 R2	+	+	+	-	+	+	+	+	-	-	+	+	-	+	+	+
	GT 12 R3	-	+	+	-	+	+	+	+	-	-	+	+	-	+	+	+
	GT 13 R1	-	+	+	-	+	+	-	+	-	+	+	+	-	+	+	+
	GT 13 R2	-	+	+	-	+	+	-	+	-	-	+	+	-	+	+	+
	GT 13 R3	-	+	+	-	+	+	-	+	-	+	+	+	-	+	+	+
	GT 14 R1	-	+	+	-	+	+	-	+	-	-	+	+	-	+	+	+

GT 14 R2	-	+	+	-	+	+	-	+	-	+	+	+	-	+	+	+
GT 14 R3	-	+	+	-	+	+	-	+	-	+	+	+	-	+	+	+
GT 15 R1	-	-	+	-	+	+	-	+	-	-	+	+	-	+	+	+
GT 15 R2	-	-	+	-	+	+	-	+	-	-	+	+	-	+	+	+
GT 15 R3	-	-	+	-	+	+	-	+	-	-	+	+	-	+	+	+
GT 16 R1	-	-	+	-	+	+	-	+	-	-	+	+	-	+	+	+
GT 16 R2	-	-	+	-	+	+	-	-	-	-	+	+	-	+	+	+
GT 16 R3	-	-	+	-	+	+	-	+	-	-	+	+	-	+	+	+
GT 17 R1	+	-	+	-	+	+	-	+	-	-	+	+	-	+	+	+
GT 17 R2	+	-	+	-	+	+	-	+	-	-	+	+	-	+	+	+
GT 17 R3	-	-	+	-	+	+	-	+	-	-	+	+	-	+	+	+
GT 18 R1	-	-	+	-	+	+	-	+	-	+	+	+	-	+	+	+
GT 18 R2	-	-	+	-	+	+	-	+	-	+	+	+	-	+	+	+
GT 18 R3	-	-	+	-	+	+	-	+	-	+	+	+	-	+	+	+
GT 19 R1	+	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 19 R2	+	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 19 R3	+	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 20 R1	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+
GT 20 R2	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+
GT 20 R3	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+
GT 21 R1	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+	+
GT 21 R2	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+	+
GT 21 R3	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+	+
GT 22 R1	+	+	+	-	+	+	+	+	-	+	+	-	-	+	+	+
GT 22 R2	+	+	+	-	+	+	+	+	-	+	+	-	-	+	+	+
GT 22 R3	+	+	+	-	+	+	+	+	-	+	+	-	-	+	+	+
GT 23 R1	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
GT 23 R2	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
GT 23 R3	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
GT 24 R1	-	+	+	-	-	+	-	+	-	+	+	-	-	-	+	+
GT 24 R2	-	+	+	-	+	+	-	+	-	+	+	-	-	+	+	+
GT 24 R3	-	+	+	-	+	+	-	+	-	+	+	-	-	+	+	+
GT 25 R1	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 25 R2	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 25 R3	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 26 R1	-	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 26 R2	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 26 R3	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 27 R1	+	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 27 R2	+	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 27 R3	-	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 28 R1	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 28 R2	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 28 R3	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 29 R1	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
GT 29 R2	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
GT 29 R3	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
GT 30 R1	+	+	+	-	+	+	+	+	-	-	+	-	-	-	+	+
GT 30 R2	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+
GT 30 R3	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+
GT 31 R1	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+
GT 31 R2	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+
GT 31 R3	+	+	+	-	+	+	+	+	-	-	+	-	-	-	+	+
GT 32 R1	+	+	+	-	-	+	+	+	-	-	+	-	-	-	+	+
GT 32 R2	-	+	+	-	-	+	+	+	-	-	+	-	-	-	+	+
GT 32 R3	+	+	+	-	-	+	-	+	-	-	+	-	-	-	+	+
GT 33 R1	+	+	+	-	+	+	-	+	-	-	+	+	-	+	+	+
GT 33 R2	+	+	+	-	+	+	-	+	-	+	+	+	-	+	+	+
GT 33 R3	+	+	+	-	+	+	-	+	-	+	+	+	-	+	+	+
GT 34 R1	+	+	+	-	+	+	-	+	-	+	+	-	-	+	+	+
GT 34 R2	+	+	+	-	+	+	-	+	-	-	+	-	-	+	+	+
GT 34 R3	+	+	+	-	+	+	-	+	-	+	+	-	-	+	+	+
GT 35 R1	-	+	+	-	-	+	-	+	-	+	+	+	-	+	+	+
GT 35 R2	-	+	+	-	-	+	-	+	-	+	+	+	-	+	+	+
GT 35 R3	-	+	+	-	-	+	-	+	-	+	+	+	-	+	+	+
GT 36 R1	-	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 36 R2	-	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 36 R3	-	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 37 R1	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 37 R2	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 37 R3	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+
GT 38 R1	-	-	+	-	-	+	-	+	-	+	+	-	-	+	+	+
GT 38 R2	-	-	+	-	-	+	-	+	-	+	+	-	-	+	+	+
GT 38 R3	-	-	+	-	-	+	-	+	-	+	+	-	-	+	+	+

Figure S1. Butterfly plots for MS^2 spectra from reference compounds

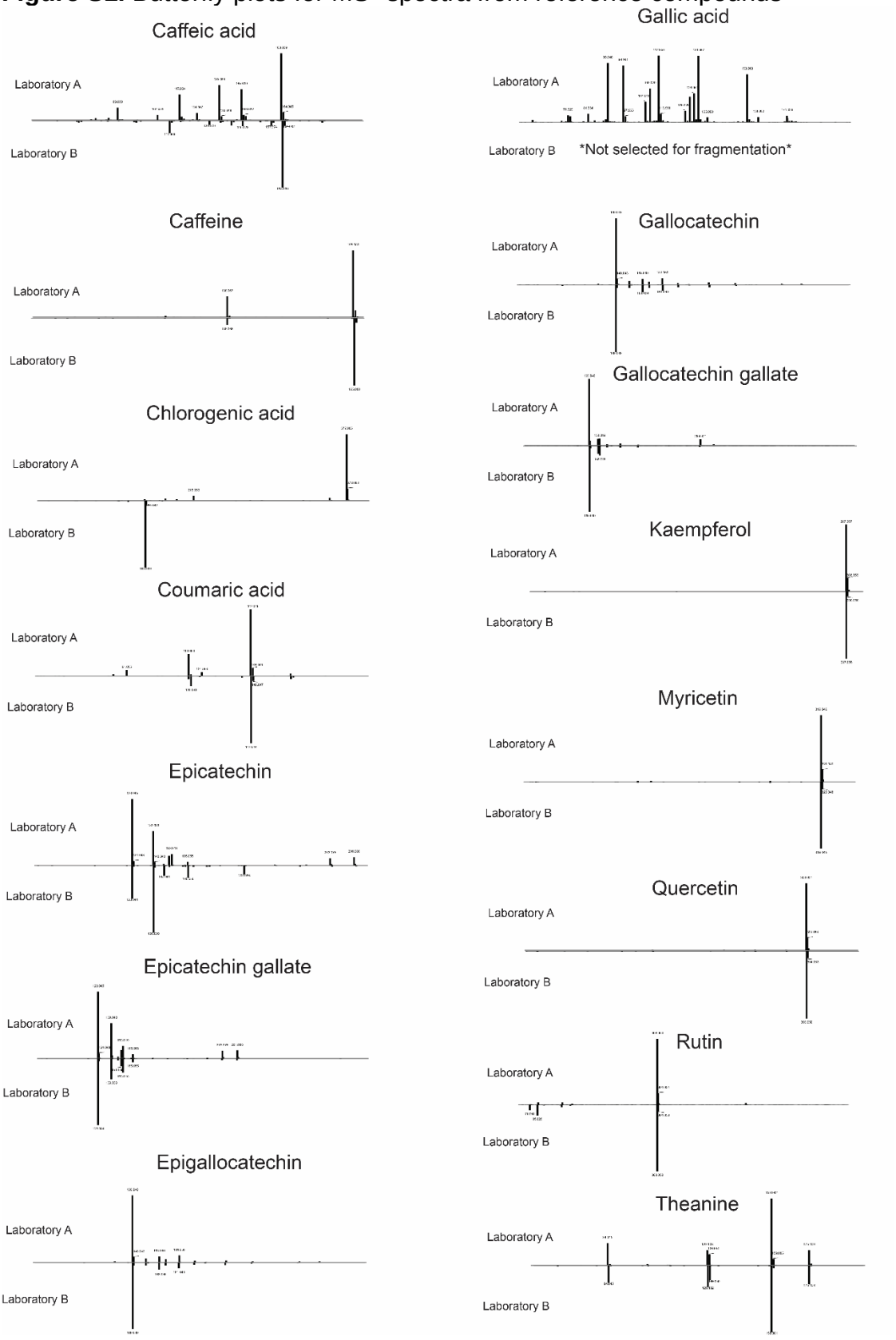


***Table S4a**:* Detailed annotations of the reference compounds and associated features for Laboratory A used for Figure 5

m/z	retention time (min)	annotation
181.0503	2.32	Caffeic acid: $[M+H]^+$
233.9608	2.30	Caffeic acid: unknown feature
251.9715	2.30	Caffeic acid: unknown feature
275.9945	2.32	Caffeic acid: unknown feature
331.9382	2.32	Caffeic acid: unknown feature
415.0102	2.32	Caffeic acid: unknown feature
194.0804	2.16	Caffeine: $[M\cdot]^+$
195.0887	2.12	Caffeine: $[M+H]^+$
195.2075	2.16	Caffeine: unknown feature
197.0928	2.16	Caffeine: unknown feature
214.0613	2.16	Caffeine: unknown feature
214.5629	2.16	Caffeine: unknown feature
223.0668	2.16	Caffeine: unknown feature
234.5747	2.16	Caffeine: unknown feature
255.0665	2.16	Caffeine: unknown feature
267.0178	2.16	Caffeine: unknown feature
295.013	2.16	Caffeine: unknown feature
311.1016	2.17	Caffeine: unknown feature
311.6031	2.16	Caffeine: unknown feature
312.0145	2.16	Caffeine: unknown feature
331.0123	2.17	Caffeine: unknown feature
346.9845	2.17	Caffeine: unknown feature
506.0959	2.16	Caffeine: unknown feature
706.0835	2.16	Caffeine: unknown feature
163.0397	1.99	Chlorogenic acid: $[M+H-C_7H_{12}O_6]^+$
354.0932	1.98	Chlorogenic acid: $[M\cdot]^+$
355.1029	1.99	Chlorogenic acid: $[M+H]^+$
337.0923	1.99	Chlorogenic acid: $[M+H-H_2O]^+$
361.1105	1.99	Chlorogenic acid: $[M+Li]^+$
377.0845	1.99	Chlorogenic acid: $[M+Na]^+$
197.0286	1.99	Chlorogenic acid: unknown feature
217.5419	1.99	Chlorogenic acid: unknown feature
228.9808	1.99	Chlorogenic acid: unknown feature
257.9851	1.99	Chlorogenic acid: unknown feature
270.0055	1.99	Chlorogenic acid: unknown feature
374.0756	1.99	Chlorogenic acid: unknown feature
393.0557	1.99	Chlorogenic acid: unknown feature
408.0139	1.99	Chlorogenic acid: unknown feature
409.0191	1.82	Chlorogenic acid: unknown feature
439.0534	1.99	Chlorogenic acid: unknown feature
449.0404	1.99	Chlorogenic acid: unknown feature

454.0193	1.99	Chlorogenic acid: unknown feature
165.0554	2.79	Coumaric acid & rutin: $[M+H]^+$
611.1606	2.79	Coumaric acid & rutin: $[M+H]^+$
303.0503	2.77	Coumaric acid & rutin: $[M+H-(C_{12}H_{22}O_{10}-H_2O)]^+$
285.0391	2.79	Coumaric acid & rutin: $[M+H-(C_{12}H_{22}O_{10}-H_2O)-H_2O]^+$
325.0577	2.79	Coumaric acid & rutin: $[M+H-(C_{12}H_{22}O_{10}-H_2O)-H_2O+ACN]^+$
257.0443	2.79	Coumaric acid & rutin: $[M+H-(C_{12}H_{22}O_{10}-H_2O)-H_2O-CO]^+$
465.1028	2.79	Coumaric acid & rutin: $[M+H-(C_6H_{12}O_5-H_2O)]^+$
449.1076	2.79	Coumaric acid & rutin: $[M+H-(C_6H_{12}O_6-H_2O)]^+$
617.1688	2.79	Coumaric acid & rutin: $[M+Li]^+$
633.1419	2.79	Coumaric acid & rutin: $[M+Na]^+$
325.5595	2.79	Coumaric acid & rutin: unknown feature
382.0142	2.79	Coumaric acid & rutin: unknown feature
397.9959	2.79	Coumaric acid & rutin: unknown feature
407.0808	2.79	Coumaric acid & rutin: unknown feature
518.0143	2.79	Coumaric acid & rutin: unknown feature
546.6025	2.79	Coumaric acid & rutin: unknown feature
631.1339	2.79	Coumaric acid & rutin: unknown feature
635.1448	2.79	Coumaric acid & rutin: unknown feature
665.0782	2.79	Coumaric acid & rutin: unknown feature
291.0865	2.37	Epicatechin: $[M+H]^+$
165.0554	2.37	Epicatechin: $[M+H-C_6H_6O_3]^+$
313.0683	2.37	Epicatechin: $[M+Na]^+$
169.0504	2.37	Epicatechin: unknown feature
174.0266	2.37	Epicatechin: unknown feature
248.0007	2.37	Epicatechin: unknown feature
249.0763	2.37	Epicatechin: unknown feature
343.9981	2.37	Epicatechin: unknown feature
345.005	2.37	Epicatechin: unknown feature
385.0248	2.37	Epicatechin: unknown feature
393.2086	2.34	Epicatechin: unknown feature
399.0382	2.32	Epicatechin: unknown feature
443.0978	2.91	Epicatechin gallate: [M+H]+
291.0867	2.90	Epicatechin gallate: $[M+H-(C_7H_6O_5-H_2O)]^+$
273.0758	2.91	Epicatechin gallate : $[M+H-(C_7H_6O_5-H_2O)-H_2O]^+$
250.0024	2.90	Epicatechin gallate: unknown feature
272.0678	2.90	Epicatechin gallate: unknown feature
345.0056	2.91	Epicatechin gallate: unknown feature
496.0085	2.89	Epicatechin gallate: unknown feature
537.0357	2.89	Epicatechin gallate: unknown feature
579.0211	2.90	Epicatechin gallate: unknown feature
938.0975	2.91	Epicatechin gallate: unknown feature
307.0816	1.92	Epigallocatechin: $[M+H]^+$
181.0502	1.92	Epigallocatechin: $[M+H-C_6H_6O_3]^+$

173.0186	1.92	Epigallocatechin: unknown feature
206.9966	1.92	Epigallocatechin: unknown feature
349.0499	1.92	Epigallocatechin: unknown feature
401.0193	1.92	Epigallocatechin: unknown feature
171.0294	1.09	Gallic acid: $[M+H]^+$
250.0027	1.09	Gallic acid: unknown feature
262.9723	1.08	Gallic acid: unknown feature
264.9673	1.09	Gallic acid: unknown feature
265.973	1.09	Gallic acid: unknown feature
266.9751	1.09	Gallic acid: unknown feature
301.9548	1.09	Gallic acid: unknown feature
321.9175	1.09	Gallic acid: unknown feature
322.9235	1.09	Gallic acid: unknown feature
394.9682	1.09	Gallic acid: unknown feature
307.0815	1.55	Gallocatechin: $[M+H]^+$
289.0705	1.55	Gallocatechin: $[M+H-H_2O]^+$
169.0505	1.54	Gallocatechin: unknown feature
173.5201	1.55	Gallocatechin: unknown feature
349.05	1.55	Gallocatechin: unknown feature
359.9924	1.55	Gallocatechin: unknown feature
399.0215	1.55	Gallocatechin: unknown feature
401.0192	1.55	Gallocatechin: unknown feature
457.9694	1.55	Gallocatechin: unknown feature
666.066	1.54	Gallocatechin: unknown feature
459.0924	2.50	Gallocatechin gallate: $[M+H]^+$
289.035	2.48	Gallocatechin gallate: $[M+H-C_8H_8O_4-2H^{app}]^+$
481.074	2.52	Gallocatechin gallate: $[M+Na]^+$
361.0004	2.51	Gallocatechin gallate: unknown feature
512.0035	2.50	Gallocatechin gallate: unknown feature
553.0305	2.52	Gallocatechin gallate: unknown feature
286.0468	4.55	Kaempferol: $[M\cdot]^+$
259.0588	4.56	Kaempferol: $[M.-CO]^+$
287.0554	4.55	Kaempferol: $[M+H]^+$
241.0499	4.56	Kaempferol: $[M+H-HCOOH]^+$
163.0049	4.58	Kaempferol: unknown feature
183.5184	4.55	Kaempferol: unknown feature
229.0503	4.55	Kaempferol: unknown feature
287.1998	4.55	Kaempferol: unknown feature
325.0021	4.56	Kaempferol: unknown feature
366.0286	4.56	Kaempferol: unknown feature
371.0074	4.57	Kaempferol: unknown feature
380.0038	4.58	Kaempferol: unknown feature
382.001	4.55	Kaempferol: unknown feature
384.0016	4.55	Kaempferol: unknown feature

387.9951	4.57	Kaempferol: unknown feature
422.9774	4.57	Kaempferol: unknown feature
438.9512	4.55	Kaempferol: unknown feature
611.0495	4.55	Kaempferol: unknown feature
626.014	4.55	Kaempferol: unknown feature
709.0239	4.55	Kaempferol: unknown feature
319.0454	3.45	Myricetin: $[M+H]^+$
178.9998	3.45	Myricetin: unknown feature
188.0057	3.45	Myricetin: unknown feature
199.5135	3.45	Myricetin: unknown feature
207.4997	3.45	Myricetin: unknown feature
322.0529	3.45	Myricetin: unknown feature
356.9911	3.45	Myricetin: unknown feature
389.9675	3.45	Myricetin: unknown feature
398.0178	3.45	Myricetin: unknown feature
413.9907	3.45	Myricetin: unknown feature
689.9933	3.45	Myricetin: unknown feature
303.0508	4.02	Quercetin: $[M+H]^+$
229.0502	4.02	Quercetin: $[M+H-CO-CO_2]^+$
285.0397	4.02	Quercetin: $[M+H-H_2O]^+$
257.0446	4.02	Quercetin: $[M+H-H_2O-CO]^+$
180.008	4.01	Quercetin: unknown feature
198.9982	4.02	Quercetin: unknown feature
200.0037	4.02	Quercetin: unknown feature
201.0552	4.02	Quercetin: unknown feature
303.1993	4.02	Quercetin: unknown feature
303.2879	4.02	Quercetin: unknown feature
305.0558	4.02	Quercetin: unknown feature
340.997	4.02	Quercetin: unknown feature
382.0232	4.02	Quercetin: unknown feature
397.9958	4.02	Quercetin: unknown feature
399.9975	4.02	Quercetin: unknown feature
454.9467	4.02	Quercetin: unknown feature
629.0511	4.02	Quercetin: unknown feature
643.0385	4.03	Quercetin: unknown feature
658.0037	4.02	Quercetin: unknown feature
755.9795	4.02	Quercetin: unknown feature
175.1086	0.49	Theanine: $[M+H]^+$
160.0867	0.49	Theanine: $[M+H-CH_3]^+$
306.0977	0.49	Theanine: unknown feature

Table S4b: Detailed annotations of the reference compounds and associated features for Laboratory B used for Figure 5

m/z	retention time (min)	annotation
181.0498	2.47	Caffeic acid: $[M+H]^+$
222.9983	2.47	Caffeic acid: $[M+H+2Na-2H]^+$
225.014	2.47	Caffeic acid: $[M+Na+Na-H]^+$
293.0001	2.47	Caffeic acid: $[M+Na+Na-H+NaCOOH]^+$
301.0484	2.47	Caffeic acid: unknown feature
304.995	2.47	Caffeic acid: unknown feature
306.0283	2.48	Caffeic acid: unknown feature
311.0079	2.47	Caffeic acid: unknown feature
316.014	2.48	Caffeic acid: unknown feature
317.0211	2.47	Caffeic acid: unknown feature
319.0215	2.48	Caffeic acid: unknown feature
319.0593	2.47	Caffeic acid: unknown feature
321.0165	2.48	Caffeic acid: unknown feature
328.0098	2.47	Caffeic acid: unknown feature
334.0236	2.47	Caffeic acid: unknown feature
342.0746	2.47	Caffeic acid: unknown feature
343.9815	2.47	Caffeic acid: unknown feature
345.9837	2.48	Caffeic acid: unknown feature
347.0542	2.47	Caffeic acid: unknown feature
354.971	2.47	Caffeic acid: unknown feature
370.9441	2.47	Caffeic acid: unknown feature
387.9475	2.47	Caffeic acid: unknown feature
422.9562	2.47	Caffeic acid: unknown feature
428.976	2.47	Caffeic acid: unknown feature
194.0793	2.33	Caffeine: $[M\cdot]^+$
195.0871	2.33	Caffeine: $[M+H]^+$
355.1012	2.17	Chlorogenic acid: $[M+H]^+$
372.1292	2.17	Chlorogenic acid: $[M+H+NH_3]^+$
353.0854	2.17	Chlorogenic acid: $[M+H-2H^{app}]^+$
370.1121	2.17	Chlorogenic acid: $[M+H-2H^{app}+NH_3]^+$
163.0386	2.17	Chlorogenic acid: $[M+H-C_7H_{12}O_6]^+$
377.0832	2.17	Chlorogenic acid: $[M+Na]^+$
399.0653	2.17	Chlorogenic acid: $[M+Na+Na-H]^+$
467.0544	2.17	Chlorogenic acid: $[M+Na+Na-H+NaCOOH]^+$
375.067	2.17	Chlorogenic acid: $[M+Na-2H^{app}]^+$
465.0363	2.17	Chlorogenic acid: $[M+Na-2H^{app}+Na-H+NaCOOH]^+$
378.0885	2.17	Chlorogenic acid: unknown feature
439.0548	2.17	Chlorogenic acid: unknown feature
454.0183	2.17	Chlorogenic acid: unknown feature
459.0226	2.18	Chlorogenic acid: unknown feature
472.0281	2.17	Chlorogenic acid: unknown feature

478.0669	2.18	Chlorogenic acid: unknown feature
480.0804	2.17	Chlorogenic acid: unknown feature
490.0646	2.16	Chlorogenic acid: unknown feature
493.0304	2.17	Chlorogenic acid: unknown feature
529.0245	2.17	Chlorogenic acid: unknown feature
533.0224	2.16	Chlorogenic acid: unknown feature
165.0543	2.96	Coumaric acid : $[M+H]^+$
209.018	2.97	Coumaric acid : $[M+Na+Na-H]^+$
277.0038	2.96	Coumaric acid : $[M+Na+Na-H+NaCOOH]^+$
318.0294	2.96	Coumaric acid : $[M+Na+Na-H+NaCOOH+ACN]^+$
244.0268	2.96	Coumaric acid : unknown feature
250.045	2.97	Coumaric acid : unknown feature
270.9889	2.97	Coumaric acid : unknown feature
285.0538	2.97	Coumaric acid : unknown feature
288.999	2.97	Coumaric acid : unknown feature
290.0336	2.97	Coumaric acid : unknown feature
295.0131	2.96	Coumaric acid : unknown feature
301.0256	2.97	Coumaric acid : unknown feature
303.0272	2.97	Coumaric acid : unknown feature
303.0647	2.96	Coumaric acid : unknown feature
304.9976	2.97	Coumaric acid : unknown feature
305.0228	2.96	Coumaric acid : unknown feature
308.0434	2.96	Coumaric acid : unknown feature
312.0141	2.97	Coumaric acid : unknown feature
326.0803	2.96	Coumaric acid : unknown feature
327.9871	2.96	Coumaric acid : unknown feature
329.9874	2.97	Coumaric acid : unknown feature
331.0589	2.97	Coumaric acid : unknown feature
338.9758	2.96	Coumaric acid : unknown feature
343.9573	2.97	Coumaric acid : unknown feature
344.9938	2.97	Coumaric acid : unknown feature
354.9486	2.96	Coumaric acid : unknown feature
406.9635	2.96	Coumaric acid : unknown feature
422.9358	2.96	Coumaric acid : unknown feature
423.0386	2.97	Coumaric acid : unknown feature
443.0959	3.04	Epicatechin gallate: $[M+H]^+$
578.0646	3.04	Epicatechin gallate: $[M+H+2xNaCOOH]^+$
273.0761	3.04	Epicatechin gallate: $[M+H-C_7H_5O_5-H_2O]^+$
487.0582	3.03	Epicatechin gallate: $[M+Na+Na-H]^+$
458.11	3.04	Epicatechin gallate: unknown feature
474.1017	3.05	Epicatechin gallate: unknown feature
492.1131	3.05	Epicatechin gallate: unknown feature
527.0505	3.04	Epicatechin gallate: unknown feature
533.0689	3.04	Epicatechin gallate: unknown feature

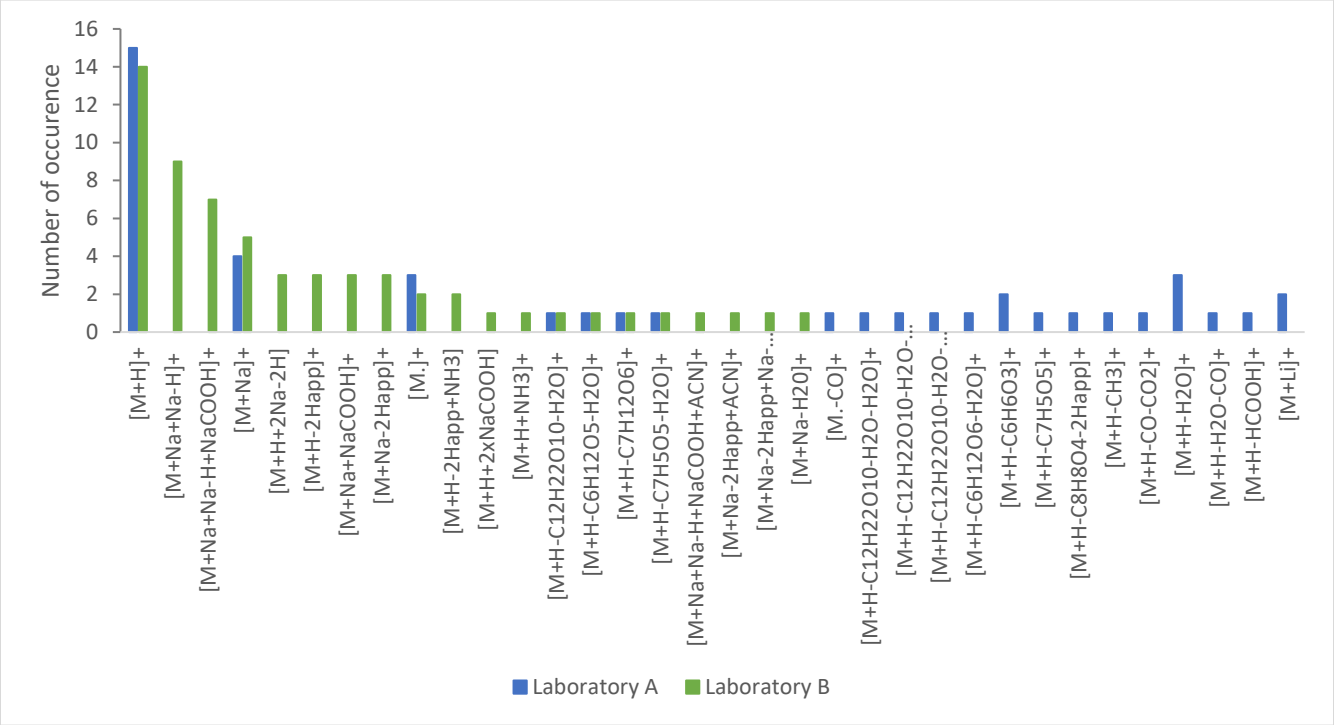
534.0683	3.05	Epicatechin gallate: unknown feature
537.0358	3.04	Epicatechin gallate: unknown feature
545.0618	3.04	Epicatechin gallate: unknown feature
549.0291	3.04	Epicatechin gallate: unknown feature
555.0506	3.04	Epicatechin gallate: unknown feature
556.0503	3.05	Epicatechin gallate: unknown feature
568.0738	3.04	Epicatechin gallate: unknown feature
590.0579	3.04	Epicatechin gallate: unknown feature
595.0367	3.03	Epicatechin gallate: unknown feature
605.0262	3.04	Epicatechin gallate: unknown feature
617.0186	3.04	Epicatechin gallate: unknown feature
623.0365	3.05	Epicatechin gallate: unknown feature
638.9989	3.04	Epicatechin gallate: unknown feature
291.0858	2.53	Epicatechin: $[M+H]^+$
289.071	2.53	Epicatechin: $[M+H-2H^{app}]^+$
306.0968	2.53	Epicatechin: $[M+H-2H^{app}+NH_3]^+$
381.055	2.52	Epicatechin: $[M+Na+NaCOOH]^+$
403.0371	2.52	Epicatechin: $[M+Na+Na-H+NaCOOH]^+$
311.052	2.53	Epicatechin: $[M+Na-2H^{app}]^+$
352.0777	2.53	Epicatechin: $[M+Na-2H^{app}+ACN]^+$
293.0907	2.53	Epicatechin: unknown feature
375.0393	2.53	Epicatechin: unknown feature
393.0479	2.53	Epicatechin: unknown feature
416.0632	2.53	Epicatechin: unknown feature
426.0502	2.52	Epicatechin: unknown feature
443.0251	2.53	Epicatechin: unknown feature
453.0109	2.53	Epicatechin: unknown feature
454.0184	2.53	Epicatechin: unknown feature
479.9709	2.52	Epicatechin: unknown feature
480.9795	2.52	Epicatechin: unknown feature
307.0819	2.05	Epigallocatechin: $[M+H]^+$
321.0603	2.05	Epigallocatechin: unknown feature
391.0342	2.05	Epigallocatechin: unknown feature
409.0427	2.06	Epigallocatechin: unknown feature
432.0596	2.05	Epigallocatechin: unknown feature
442.0452	2.05	Epigallocatechin: unknown feature
469.0086	2.05	Epigallocatechin: unknown feature
495.966	2.05	Epigallocatechin: unknown feature
329.063	2.05	Epigallocatechin: $[M+Na]^+$
397.0505	2.06	Epigallocatechin: $[M+Na+NaCOOH]^+$
419.0327	2.05	Epigallocatechin: $[M+Na+Na-H+NaCOOH]^+$
214.9924	1.26	Gallic acid: $[M+Na+Na-H]^+$
459.0929	2.64	Gallocatechin gallate: $[M+H]^+$
481.0746	2.64	Gallocatechin gallate: $[M+Na]^+$

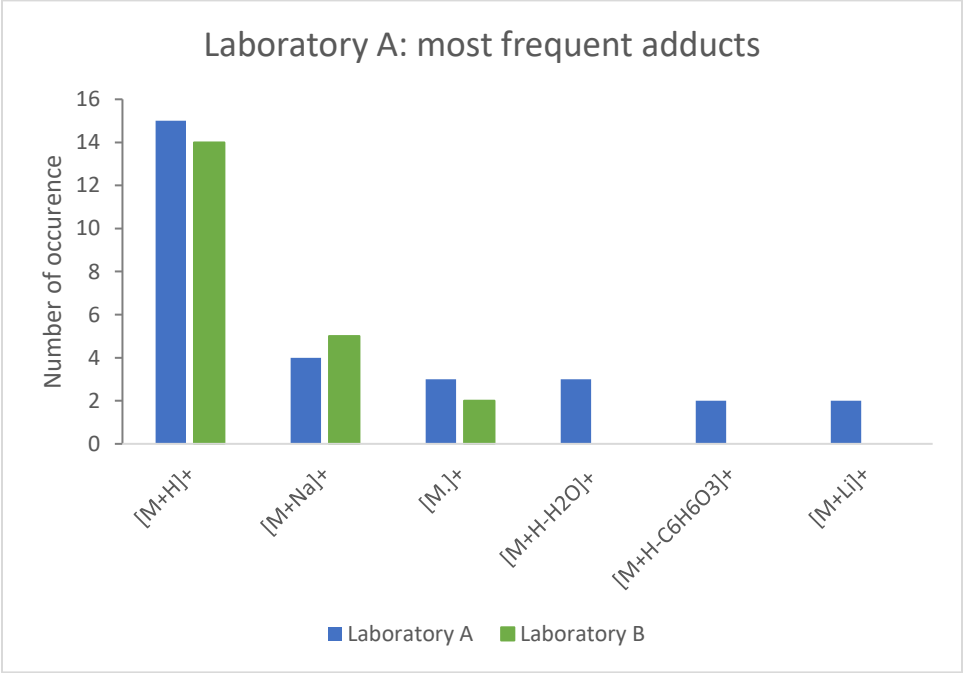
549.0601	2.63	Gallocatechin gallate: $[M+Na+NaCOOH]^+$
503.0544	2.64	Gallocatechin gallate: $[M+Na+Na-H]^+$
571.0452	2.64	Gallocatechin gallate: $[M+Na+Na-H+NaCOOH]^+$
303.0496	2.64	Gallocatechin gallate: unknown feature
461.0957	2.64	Gallocatechin gallate: unknown feature
492.1132	2.64	Gallocatechin gallate: unknown feature
508.1104	2.64	Gallocatechin gallate: unknown feature
553.0286	2.63	Gallocatechin gallate: unknown feature
584.0691	2.64	Gallocatechin gallate: unknown feature
594.0544	2.64	Gallocatechin gallate: unknown feature
595.0644	2.63	Gallocatechin gallate: unknown feature
606.0549	2.63	Gallocatechin gallate: unknown feature
307.0819	1.70	Gallocatechin: $[M+Na+Na-H+NaCOOH]^+$
286.0469	4.74	Kaempferol: $[M^{\cdot}]^+$
287.0543	4.73	Kaempferol: $[M+H]^+$
399.0044	4.74	Kaempferol: $[M+Na+Na-H+NaCOOH]^+$
407.0533	4.74	Kaempferol: unknown feature
224.5442	4.74	Kaempferol: unknown feature
289.0595	4.73	Kaempferol: unknown feature
366.0267	4.74	Kaempferol: unknown feature
384.039	4.74	Kaempferol: unknown feature
392.9885	4.74	Kaempferol: unknown feature
408.0596	4.74	Kaempferol: unknown feature
408.9602	4.74	Kaempferol: unknown feature
410.999	4.74	Kaempferol: unknown feature
412.0323	4.74	Kaempferol: unknown feature
423.0266	4.74	Kaempferol: unknown feature
425.0263	4.74	Kaempferol: unknown feature
425.0637	4.74	Kaempferol: unknown feature
425.9624	4.74	Kaempferol: unknown feature
426.9985	4.74	Kaempferol: unknown feature
427.0236	4.74	Kaempferol: unknown feature
434.0165	4.74	Kaempferol: unknown feature
435.0173	4.74	Kaempferol: unknown feature
448.082	4.74	Kaempferol: unknown feature
451.9886	4.73	Kaempferol: unknown feature
453.0612	4.75	Kaempferol: unknown feature
460.9783	4.73	Kaempferol: unknown feature
319.0459	3.60	Myricetin: $[M+H]^+$
360.9927	3.60	Myricetin: $[M+H+2Na-2H]^+$
317.0289	3.60	Myricetin: $[M+H-2H^{app}]^+$
363.0082	3.61	Myricetin: $[M+Na+Na-H]^+$
339.0115	3.60	Myricetin: $[M+Na-2H^{app}]^+$
321.0495	3.61	Myricetin: unknown feature

430.9929	3.60	Myricetin: unknown feature
439.0461	3.60	Myricetin: unknown feature
440.0469	3.61	Myricetin: unknown feature
454.0093	3.61	Myricetin: unknown feature
455.016	3.60	Myricetin: unknown feature
457.0194	3.61	Myricetin: unknown feature
457.0565	3.60	Myricetin: unknown feature
466.0037	3.61	Myricetin: unknown feature
492.9685	3.61	Myricetin: unknown feature
303.0497	4.19	Quercetin: $[M+H]^+$
347.0149	4.19	Quercetin: $[M+H+2Na-2H]^+$
382.022	4.19	Quercetin: unknown feature
397.9937	4.19	Quercetin: unknown feature
408.9835	4.19	Quercetin: unknown feature
414.9987	4.19	Quercetin: unknown feature
423.0514	4.19	Quercetin: unknown feature
424.0518	4.19	Quercetin: unknown feature
424.9556	4.19	Quercetin: unknown feature
428.0295	4.19	Quercetin: unknown feature
438.0134	4.19	Quercetin: unknown feature
439.0202	4.19	Quercetin: unknown feature
441.0243	4.19	Quercetin: unknown feature
442.994	4.19	Quercetin: unknown feature
450.0087	4.19	Quercetin: unknown feature
451.0151	4.19	Quercetin: unknown feature
464.0772	4.19	Quercetin: unknown feature
476.9734	4.19	Quercetin: unknown feature
611.1595	2.92	Rutin: $[M+H]^+$
303.0497	2.93	Rutin: $[M+H-(C_{12}H_{22}O_{10}-H_2O)]^+$
465.1029	2.92	Rutin: $[M+H-(C_6H_{12}O_5-H_2O)]^+$
633.142	2.92	Rutin: $[M+Na]^+$
655.1217	2.92	Rutin: $[M+Na+Na-H]^+$
301.0339	2.93	Rutin: unknown feature
626.1744	2.93	Rutin: unknown feature
631.1291	2.93	Rutin: unknown feature
665.0769	2.93	Rutin: unknown feature
695.116	2.93	Rutin: unknown feature
701.1252	2.92	Rutin: unknown feature
705.0952	2.92	Rutin: unknown feature
717.0972	2.92	Rutin: unknown feature
723.108	2.92	Rutin: unknown feature
393.1709	0.59	Theanine: $[2M+Na+Na-H]^+$
175.1079	0.59	Theanine: $[M+H]^+$
197.0894	0.59	Theanine: $[M+Na]^+$

219.0717	0.59	Theanine: $[M+Na+Na-H]^+$
179.0792	0.59	Theanine: $[M+Na-H_2O]^+$
168.1033	0.59	Theanine: unknown feature
176.1044	0.59	Theanine: unknown feature
177.1124	0.59	Theanine: unknown feature
189.1234	0.59	Theanine: unknown feature
208.1326	0.59	Theanine: unknown feature
231.0649	0.59	Theanine: unknown feature
254.081	0.59	Theanine: unknown feature
259.0598	0.59	Theanine: unknown feature
270.0526	0.60	Theanine: unknown feature
274.0244	0.59	Theanine: unknown feature
275.0318	0.59	Theanine: unknown feature
277.0324	0.59	Theanine: unknown feature
281.0415	0.59	Theanine: unknown feature
287.059	0.59	Theanine: unknown feature
342.0115	0.59	Theanine: unknown feature
409.1367	0.59	Theanine: unknown feature

Figure S2. Number of occurrences of adducts/fragments in the annotated features in the analyses of the mixture of reference compounds by laboratory A and laboratory B





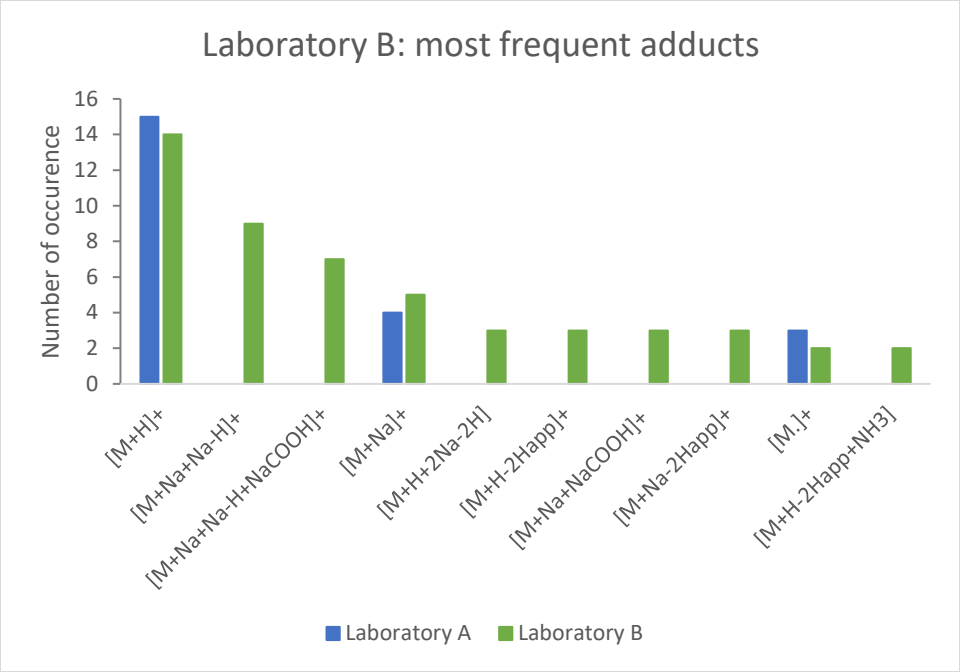

Laboratory B: most frequent adducts
Number of occurence
16
14
12
10
8
6
4
2
0
[M+H]+
[M+Na+Na-H]+
[M+Na+Na-H+NaCOOH]+
[M+Na]+
[M+H+2Na-2H]
[M+H-2Happ]+
[M+Na+NaCOOH]+
[M+Na-2Happ]+
[M.]+
[M+H-2Happ+NH3]
Laboratory A
Laboratory B

Table S5a: Reference list of charged adducts used for annotation

Charge carrier	Neutral mass	Formal annotation	Mass difference related to [M+H]+	Comment	References
H^+	NH_3-H_2O	$[M+H+NH_3-H_2O]^+$	-0.98456		4
Li^+		$[M+Li]^+$	6.00763		4
H^+	NH_3	$[M+H+NH_3]^+$	17.02600		5
H^+	H_2O	$[M+H+H_2O]^+$	18.01002		4
Na^+		$[M+Na]^+$	21.98140		5
H^+	CH_3OH	$[M+H+CH_3OH]^+$	32.02567	methanol	5
Na^+	NH_3	$[M+Na+NH_3]^+$	39.00795		6
Na^+	H_2O	$[M+Na+H_2O]^+$	39.99196		5
H^+	CH_3CN	$[M+H+CH_3CN]^+$	41.02600	acetonitrile	5
Na+	Na-H	$[M+Na+Na-H]^+$	43.96334		5
H^+	HCOOH	$[M+H+HCOOH]^+$	46.00493		7
K^+	NH_3	$[M+K+NH_3]^+$	54.98188		6
H^+	NaCl	$[M+H+NaCl]^+$	57.95807		6
Na^+	K-H	$[M+Na+K-H]^+$	59.93728		6
H^+	C_3H_8O	$[M+H+C_3H_8O]^+$	60.05697	isopropanol	5
Na^+	CH_3CN	$[M+Na+CH_3CN]^+$	63.00795	acetonitrile	5
H^+	NH_3+HCOOH	$[M+H+NH_3+HCOOH]^+$	63.03148		7
Na^+	2Na-2H	$[M+Na+2Na-2H]^+$	65.94529		5
Na^+	HCOOH	$[M+Na+HCOOH]^+$	67.98688		7
K^+	K-H	$[M+K+K-H]^+$	75.91122		5
K^+		$[M+K]^+$	37.95533		5
H^+	C_2H_6OS	$[M+H+C_2H_6OS]^+$	78.01339	DMSO	5
K^+	CH_3CN	$[M+K+CH_3CN]^+$	78.98188	acetonitrile	6
Na^+	NaCl	$[M+Na+NaCl]^+$	79.94002		6
H^+	2xCH_3CN	$[M+H+2xCH_3CN]^+$	82.05255	acetonitrile	5
H^+	C_3H_8O+Na	$[M+H+C_3H_8O+Na]^+$	83.04674	isopropanol	5
K^+	HCOOH	$[M+K+HCOOH]^+$	83.96081		7
Na^+	NaCOOH	$[M+Na+NaCOOH]^+$	89.96882		6
K^+	NaCl	$[M+K+NaCl]^+$	95.91396		6
K^+	NaCOOH	$[M+K+NaCOOH]^+$	105.94276		6
Na^+	Na-H+NaCOOH	$[M+Na+Na-H+NaCOOH]^+$	111.95077		Observed in Lab A dataset
H^+	Na-H+NaCOOH	$[M+H+Na-H+NaCOOH]^+$	89.96882		Observed in Lab A dataset
H^+	CH_3OH-H_2O	$[M+H+CH_3OH-H_2O]^+$	14.01510		4
H^+	H_2^{app}	$[M+H+H_2^{app}]^+$	-2.01620	oxidation-like process	4

Table S5b: Reference list of neutral losses used for annotation

Neutral losses			
Description	**Mass difference**	**Comments**	**Reference**
($C_6H_{12}O_5$-H_2O)	-146.05791	desoxy-hexose-H_2O / methyl-pentose-H_2O	8
($C_{12}H_{22}O_{10}$-H_2O)	-308.11074	rutinose-H_2O	8
2xH_2O	-36.02113		9
C_2H_6O	-46.04187		9
3xH_2O	-54.03170		9
CH_3-COOH	-60.02113		9
CO-CO_2	-74.00040		9
CH_3-CH_2-COOH-NH	-89.04768		9
2xCO_2-H_2O	-105.99023		9
2CO_2+2H_2O	-124.00079		9
$C_6H_8O_6$	-176.03209	glucuronic acid	4
NH_3	-17.02655	amine containing molecule	4
H_2O	-18.01057		4
H_2O-NH_3	-35.03711		4
C_2H_2O	-42.01057	deacetylation	4
2Na-2H	-43.96389		4
$C_9H_8O_3$-H_2O	-146.03678	coumaroyl	4
($C_6H_{12}O_6$-H_2O)	-162.05283	hexose-H2O	4
$C_6H_6O_3$	-126.03170	Hetero Cyclic Ring Fragmentation	10
$C_8H_8O_2$	-136.05243	Retro Diels Alder (ring B:1xOH)	10
$C_8H_8O_3$	-152.04735	Retro Diels Alder (ring B: 2xOH)	10
$C_8H_8O_4$	-168.04226	Retro Diels Alder (ring B: 3xOH)	10
$C_6H_6O_3$-H_2O	-144.04226	Hetero Cyclic Ring Fragmentation-H2O	10
$CH_3^{\bullet}$	-15.02348	demethylation	11
O	-15.99492		11
CHN	-27.01090		11
CO	-27.99492		11
CH_3OH	-32.02622	methanol	11
CO_2	-43.98983		11
COOH	-44.99766		11
HCOOH	-46.00548		11
2xCO	-55.98983		11
($C_7H_6O_5$-H_2O)	-152.01096	galloyl	12
NaCOOH	-67.98743		6
$C_7H_{12}O_6$	-192.06339	quinic acid	13

Table S5c: Reference list of neutral additions used for annotation

Neutral additions			
Description	**Mass difference**	**Comments**	**Reference**
NaCl	57.95862		[9]
Na+C_3H_8O	83.04729	sodium and isopropanol	[5]
KCOOH	83.96136		[9]
NaCOOH+Na-H	89.96937		[9]
2xNaCl	115.91725		[9]
2xNaCOOH	135.97485		[9]
Na-H	21.98195		[9]
K-H	37.95588		[9]
C_2H_3N	41.02655	acetonitrile	[9]
3Na-3H	65.94584		[9]
HCOOH+NaCOOH	113.99291		[9]
NaCl+NaCOOH	125.94605		[9]
CH_3OH	32.02622	methanol	[4]
HCOOH	46.00548		[4]
C_3H_8O	60.05752	isopropanol	[5]

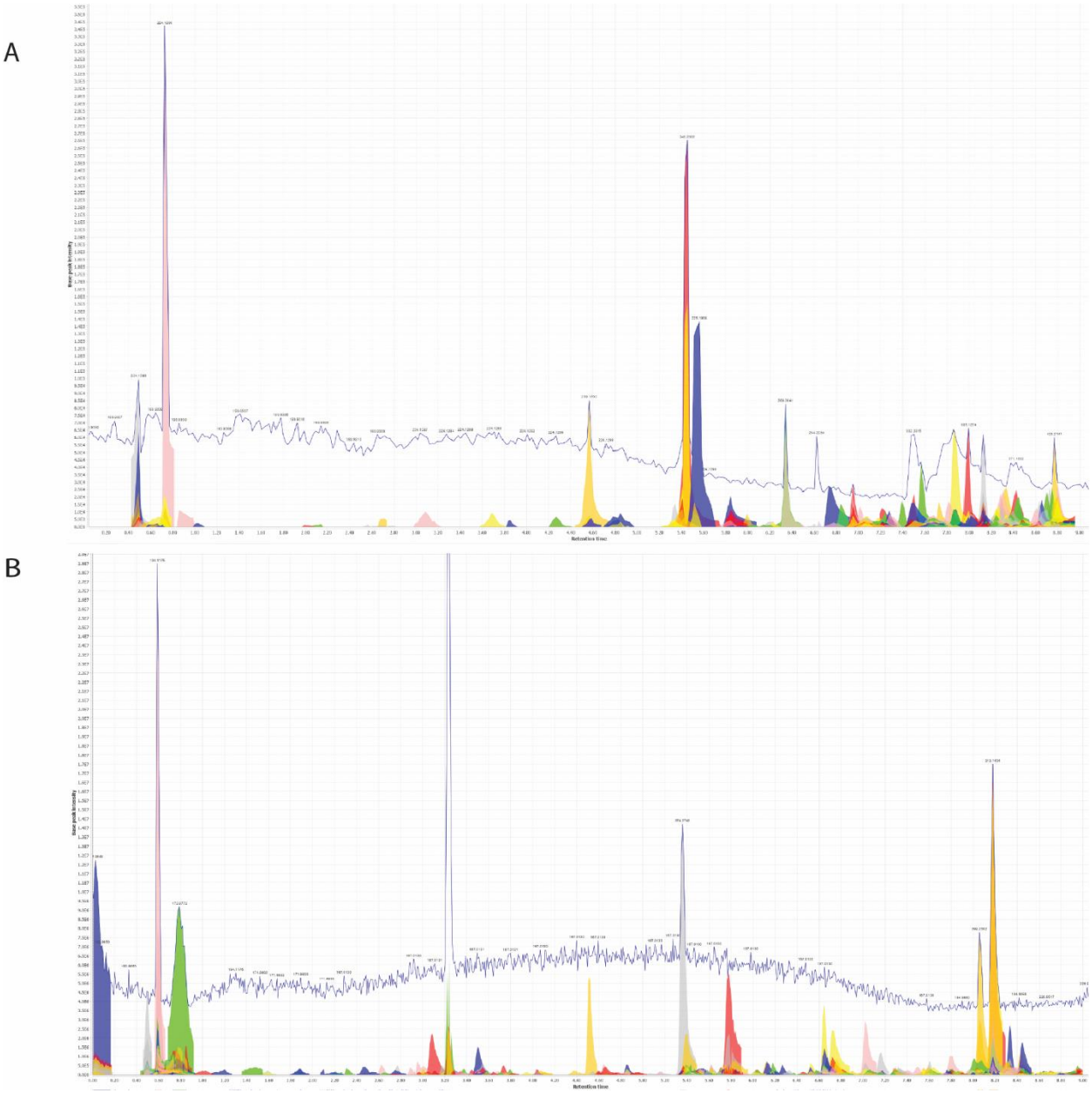


Figure S3. Blank traces for Laboratory A (**A**) and Laboratory B (**B**) showing TIC (above in blue line) and retained features after MZmine 2.0 processing (below).

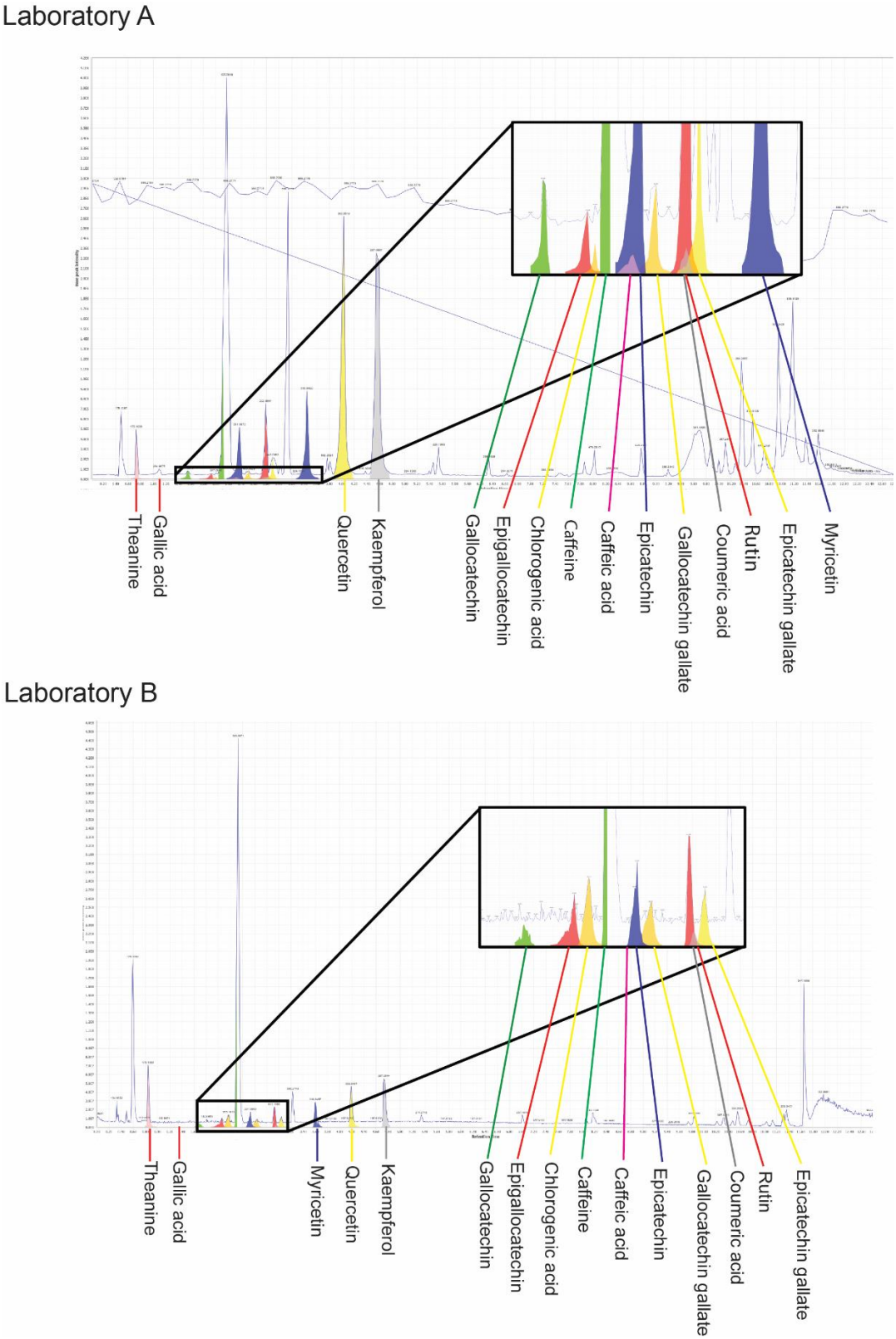


Figure S4. Standard mix injection traces for Laboratory A and Laboratory B after MZmine 2.0 processing and filtered for only the standards.

Table S6. Comparison of limits of detection for three representative green tea constituents between Laboratory A and Laboratory B.

Compound	Laboratory A[a]		Laboratory B[b]	
	LOD (µg/mL)[c]	LOQ (µg/mL)[d]	LOD (µg/mL)	LOQ (µg/mL)
(-)-epigallocatechin	0.57	1.9	0.078	0.26
rutin	0.091	0.30	0.044	0.15
kaempferol	0.25	0.84	0.061	0.20

a. Data for laboratory A were collected on a Waters SYNAPT G2-Si qTOF mass spectrometer.
b. Data for laboratory B were collected with a Thermo Fisher Q-Exactive Plus Orbitrap.
c. Limit of detection (LOD) was calculated from analyzing standards in triplicate at a range of concentrations between 0.2 and 100 µg/mL on each instrument. Only the linear portion of the calibration curve was used for the LOD determination. LOD was calculated by the equation $LOD = \frac{3s}{m}$, where m is the slope of the calibration curve and s = the standard deviation in peak area of three replicate injections of the standard at the lowest concentration within the linear range of the calibration curve.
d. Limit of Quantitation (LOQ) was calculated by the equation $LOD = \frac{10s}{m}$.

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