International Journal of Mass Spectrometry 468 (2021) 116663

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Exploratory analysis using MRM profiling mass spectrometry of a candidate metabolomics sample for testing system suitability

Madison E. Edwards [a], César A. Marasco Jr. [a, b], Tracey B. Schock [c], Tiago J.P. Sobreira [d], Christina R. Ferreira [a, d], R. Graham Cooks [a, d, *]

[a] Department of Chemistry, Purdue University, West Lafayette, IN, 47907, United States
[b] Department of Analytical Chemistry, Sao Paulo State University, Araraquara, Sao Paulo, 14801, Brazil
[c] National Institute of Standards and Technology, Chemical Sciences Division, Charleston, SC, 29412, United States
[d] Bindley Bioscience Center, Purdue University, West Lafayette, IN, 47907, United States

ARTICLE INFO

Article history:
Received 9 February 2021
Received in revised form
2 June 2021
Accepted 26 June 2021
Available online 29 June 2021

Keywords:
Lipidomics
Metabolomics
Exposome
Reproducibility
Tandem mass spectrometry
NIST RGM 10122

ABSTRACT

Multiple reaction monitoring (MRM) profiling is an exploratory mass spectrometry (MS) method applicable to the initial screening of complex samples for small molecules based on their chemical functionalities. We report on the applicability and quality of this method to screen for metabolites, lipids and exogenous compounds in a candidate reference material, the Metabolomics System Suitability Research Grade Material (RGM 10122) which is being developed by the National Institute of Standards and Technology (NIST). In an initial discovery experiment, we recorded data using eighty neutral loss (NL) and precursor ion (Prec) MS/MS scans, selected from literature data as likely of value in recognizing the presence of potential lipids and metabolites. Then the NIST sample was re-examined combining precursor-to-product ion transitions from the discovery experiment with a list of 1357 known literature-based metabolite MRMs. This MRM profiling experiment gave a small set (191) of high-quality lipid specific MRMs for the sample. Similar experiments gave 104 and 17 metabolite and exosome MRM's. These MRM experiments, with a few exceptions, showed relative standard deviations (RSD) under 25% for individual tentatively assigned compounds. At a data acquisition rate of 50 compounds/min, using two MRMs for each compound, this approach allows quick surveys for easily detectable compounds in complex samples.

1. Introduction

Continued progress in small molecule analysis by mass spectrometry (MS) has reinforced the need for analytical standards for biological matrices, such as biofluids and tissues. Such complex standards are necessary to support the development and validation of workflows for sample preparation, measurement, and data analysis, and are required for the evaluation of inter-laboratory comparability. One of the first such initiatives involving the use of a human biofluid analytical standard for metabolomics was taken in 2010 when the LIPID Metabolites and Pathways Strategy (LIPID MAPS) consortium published the analytical results of NIST Standard Reference Material (SRM) 1950 Metabolites in Frozen Human Plasma and quantified the abundant lipid species [1] Subsequent use of this NIST SRM contributed to a harmonization effort undertaken through an interlaboratory comparison exercise involving 31 different sites [2].

A wide range of chemical functional groups occurs in individual lipids and metabolites and they can be categorized as polar (metabolites, some lipids, and most drugs) or non-polar (some lipids) compounds. The structural variety extends even further if one considers exogenous compounds such as synthetic chemicals, drugs and their metabolites; the human metabolome can be used as a basis to study exposure to exogenous compounds and hence to provide a framework for study of exposure–response relationships [3].

Full mass scan exploratory analysis for the identification of small molecule metabolites, lipids, and exogenous compounds typically utilizes commercial platforms often including liquid-chromatography (LC) coupled to high-resolution mass spectrometers (HRMS), especially time-of-flight (ToF) and Orbitrap

* Corresponding author. Department of Chemistry, Purdue University, West Lafayette, IN, 47907, United States.
E-mail address: cooks@purdue.edu (R.G. Cooks).

https://doi.org/10.1016/j.ijms.2021.116663
1387-3806/

instruments, or hybrids like the quadrupole/ToF. Sample separation by LC is followed by product ion MS/MS scans. It is important to note that even though the product ion scan MS/MS is the most common MS/MS experiment, this is just one of the four types of MS/MS experiments that can be performed in triple quadrupoles and other types of mass spectrometers. Two other MS/MS scans, namely the precursor ion scan (Prec) and the neutral loss scan (NL), allow profiling for chemical functionalities since these frequently yield class-diagnostic product ions or neutral losses [4]. Therefore, while the product ion scan is ideal for obtaining structural information on a specific precursor ion and hence a specific compound, Prec and NL are powerful scan modes for exploratory analysis of small molecules at the level of the chemical functional group.

From these considerations, an alternative exploratory method has emerged in which Prec and NL scans are used in experiments (or alternatively taken from the literature) to discover precursor-to-product transitions which are distinctive for particular functionalities. Once these are recognized, then individual samples can be screened rapidly for these chemical signatures using a set of transitions assembled in a multiple reaction monitoring (MRM) set. This procedure, under the name MRM profiling, has been described [5] and reviewed [6]. Chromatographic separation and the use of internal standards for structural confirmation or absolute quantification are reserved for a final target validation step [7] when such a step is deemed necessary to solve the particular problem in hand. Examples of published applications of the MRM profiling method include identification of cases of drug resistance in bacteria [8], profiling of epidermal lipids in a mouse model of dermatitis [9], lipid extract stability [10] and characterization of peri-ovarian adipose tissue [11]. MRM profiling has also been integrated with proteomics in analysis of the gold nanoparticle biocorona in healthy and obese populations [12]. Structural confirmation and absolute quantification have been performed in some studies, but the main objective of the MRM profiling method is to survey the chemical composition of the sample at a higher level than individual compound identification in order to guide further exploratory and validation efforts. This approach finds particular value in the classification of samples into related groups. We envisage that the MRM profiling approach can support efforts in which MS is applied to point-of-care analysis, specially using simple instruments and low cost materials such as paper spray ionization [13,14].

In this research, the MRM profiling method was applied for the exploratory analysis of lipids, metabolites, and exogenous compounds in the NIST material currently being developed as a Metabolomics System Suitability Sample (NIST RGM 10122), which will soon become a NIST standard. This sample is a dried polar extract of human liver intended for evaluation of instrument suitability and performance in advance of a metabolomics study. In addition, the NIST liver extract sample also served to provide information on the reproducibility of the MRM methodology.

2. Materials and methods

2.1. RGM 10122 production

Liver tissues were cryo-homogenized at the NIST laboratory in Charleston (SC, USA) using established protocols [20]. Homogenized liver powder was extracted with 70% volume fraction ethanol in water and stored at −80 °C to facilitate protein precipitation. The liver sample was then centrifuged at 4 °C, the supernatant was transferred, and the centrifugation step was repeated. The final supernatant was diluted twice (2×) with 70% volume fraction ethanol in water, dispensed into autosampler vials and stored in a liquid nitrogen vapor freezer prior to drying by vacuum centrifuge. Four vials, each containing a dried extract of approximately 100 mg tissue equivalent, were provided for this study. The first vial was used in the exploratory experiments. The last three vials were used in the measurement of reproducibility.

2.2. Sample preparation

The dried NIST RGM 10122 sample extract was resuspended in 100 μL of MeOH/$CHCl_3$ (3:1 v/v) with 5 ng mL^{-1} of butylated hydroxytoluene (referred to as the stock solution). The stock solution was then diluted in acetonitrile (ACN)/methanol (MeOH)/ammonium acetate ($NH_4CH_3CO_2$) 300 mM 6.65:3:0.35 v/v/v (referred to as the injection solvent) to dilute the stock solution by hundred times (100×) for MRM profiling experiments and by fifty times (50×) for the acquisition of product ions scans.

2.3. Instrumentation and data acquisition

The experiments were performed in a triple quadrupole mass spectrometer (6410 Agilent) equipped with a capillary pump (Agilent 1100 series) and an autosampler (G1377A Agilent). Both positive and negative ion modes with an isolation window of ± 0.7 mass units were used for the experiments. A volume of 8 μL (8 μL) of the sample was used for flow injection (i.e. no chromatographic separation) in MRM profiling experiments and in the reproducibility study. For each injection, data was acquired over 2 min. The 8 μL sample volume was chosen for injection because it provides ion signal for around 45 s, which avoids carry over to the next sample injection when using 2 min of data acquisition. The volume of 12 μL chosen for the product ion scans gave higher ion signals which was needed since this scan is less sensitive than are MRM experiments. ACN containing 0.1% formic acid at the flow rate of 10 μL $min^{-1}$was the solvent delivered by the capillary pump. No solvent gradient was used.

2.4. MRM-profiling exploratory experiments

The MRM profiling workflow for sample screening (Fig. 1) used the instrumental conditions described above. Discovery step experiments included the 80 NL and Prec MS/MS scans for lipids and metabolites which are listed in Table S1. The transitions observed in the discovery step experiments were combined with a list of 1357 MRMs based on the Lipid Maps Structure Database (LMSD) for ten classes of lipids (Table S2), with 611 MRMs related to 155 metabolites commonly detected in biological samples (Table S3), and with 252 MRMs related to 90 exogenous compounds (Table S4). The literature-derived MRMs related to metabolites and exogenous compounds were compiled from the METLIN database (www.metlin.com) together with metabolite [22,23] and exogenous biofluid and tissue literature [15–30]. Data acquisition was performed at a rate of 200 MRMs during each 2 min sample injection (i.e. 100 compounds in 2 min when using two MRMs for each compound). The MRMs were acquired using the same solvent and ionization source conditions, i.e. ESI conditions without intentional adduct formation. Note that the Lipid Maps Structure Database - LMSD (www.lipidmaps.org) is a relational database encompassing structures and annotations of biologically relevant lipids. Structures of lipids in the LMSD come from several sources, including the LIPID MAPS® Consortium's core laboratories and partners, lipids identified by LIPID MAPS® experiments, biologically relevant lipids manually curated from LIPID BANK, LIPIDAT, Lipid Library, Cyberlipids, ChEBI and other public sources. Also novel lipids described in papers submitted to peer-reviewed journals, computationally generated structures for appropriate classes are included in the LMSD.

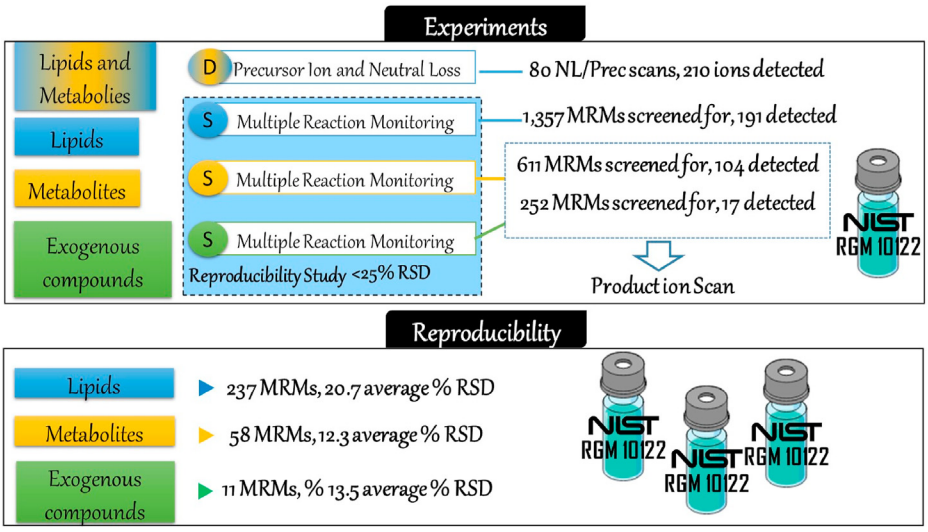

Fig. 1. Overview of the MRM profiling workflow used in this study. In the **discovery step (D)**, 80 precursor and neutral loss ion scans were used to profile compound classes and structural motifs related to lipids and metabolites, resulting in 210 MRM transitions being detected from 49 NL and Prec scans. For the **screening step (S)**, 86 transitions seen (using NL/Prec scans) in the discovery step and for which a tentative ID was found in Metlin or LipidMaps, were combined with MRMs based on class-specific parent/product ion pairs listed at the Lipid Maps database, resulting in a final list of 1357 MRMs. The NIST sample was then examined for these MRMs and it gave 191 MRM hits all related to phospholipids. Also, MRMs related to metabolites reported in Metlin or in the literature [21–23] were combined with the discovery data, and these 611 MRMs represented 155 metabolites. The NIST sample was then examined for these MRMs and it gave 104 hits. MRMs for 90 exogenous compounds (252 MRMs) were also screened and these gave 17 hits. For the reproducibility study, three vials of the sample were used to measure the % RSD of the signals for the detected lipids, metabolites, and exogenous compounds. With a few exceptions, the % relative standard deviation (RSD) for a variety of compounds as measured by their MRM transition(s) was under 20%.

2.5. Detection of expected fragments by product ion scans in metabolites and exogenous compounds

Precursor ion *m/z* values corresponding to metabolites and exposomes that showed ion intensities higher than those for the blank sample were used for the acquisition of product ion MS/MS data to obtain evidence for the tentative molecular structure attribution. Product ion MS/MS was performed using four different collision energies (10, 20, 25, and 30 V) for precursor ions of metabolites and exogenous compounds (Tables S6 and S7).

2.6. Reproducibility study

Three additional vials of NIST RGM 10122 were used for the reproducibility study. MRMs for lipids, metabolites, and exogenous compounds were applied to screen a pooled sample composed of aliquots from each of the three new vials of the NIST 10122 sample and the detected MRMs were used for % RSD calculation as described below.

Triplicate sample injections were performed for each sample vial. The measurements were run with samples either spiked or not spiked with Avanti Equisplash® Mix (#330731, Avanti Polar Lipids, Alabaster, AL). For those measurements where IS was spiked (total of 1.6 ng of each IS for each sample injection), the ion intensities of the IS's sphingomyelin (SM), phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylcholine (PC) were used to normalize the ion signals of all lipids in the same class. These classes were selected for normalization by IS since relative quantification using one or few IS to represent the whole class is generally considered acceptable. For quantitation of lipids in other classes, such as cholesteryl esters which show in-source fragmentation, matching internal standards is usually required [22,31].

2.7. Data analysis

When profiling experiments are carried out by direct sample injection (as in this research) or by LC-HRMS. Ions higher in intensity than the background are used for database search and statistical analysis. Here we applied a filter requiring ion signals to be 30% higher than for a blank sample (i.e. just the injection solvent) to refer to an ion or an MRM as detected and so to include it in the profile. Therefore, if a MRM is referred to as "detected," it means that it showed an ion intensity at least 30% higher that the value obtained for the injection solvent. For the relative quantification data, where normalization by spiked internal standards was performed, signal-to-noise threshold (S/N) $\geq$3 was used. This gave similar data to the 30% above blank method.

Ion intensities of each MRM were summed for the scans obtained over 2 min of data acquisition for the samples and for the injection solvent (blank sample) using home-built scripts. This data was used to calculate which MRMs were detected and the fold change compared to the blank sample. For the reproducibility study, relative standard deviations (% RSD) were determined using total ion counts of each MRM. The % RSD was then calculated as $\%\,RSD = \left(\frac{\mathrm{STDEV}_{\mathrm{data\ range}}}{\mathrm{AVERAGE}_{\mathrm{data\ range}}}\right) \times 100$.

3. Results and discussion

This research interrogated for selected small molecule chemical functionalities in the NIST RGM 10122 polar liver extract using an MRM profiling strategy. A clear understanding of the four MS/MS scan modes in mass spectrometry (Prec, NL, Product ion scan and MRM) as outlined by Schwartz et al. 1990 and recently reviewed [6] is necessary to draw parallels between MRM profiling and the typical exploratory methods for small molecule composition in biological samples.

The experiments reported here were not aimed at individual compound identification nor were they comprehensive since various other chemical functionalities could have been sought. Nonetheless, those selected represent common compound classes that are present as small molecules in lipids, metabolites, and exogenous compounds. The data shown were acquired in quickly,

2 min for each Prec or NL scan or 100 MRMs/min after sample dilution.

We started by interrogating the sample using 80 Pre and NL MS/MS scans (Table S1) related to lipids and metabolites and refer to this as the discovery step. That is, we use functional group selective MS/MS scans to discover MRMs related to these two classes of biological compounds. In order to screen the NIST standard sample, ion transitions (MRM scans) detected by Prec and NL for which a tentative ID found in the Metlin or the Lipid Maps database has chemical functionalities of lipids and metabolites (N = 86), were combined with additional MRMs. These additional MRMs were compiled from the Lipid Maps Structure Database (Table S2) together with MRMs compiled from the literature and the Metlin data base for metabolites (Table S3) and for exogenous compounds (Table S4). The experimental design and a summary of the outcomes is described in Fig. 1.

For the MRM profiling discovery step, we applied 80 Prec and NL MS/MS scans which were related to known structural motifs of lipids (35 MS/MS scans) and metabolites (45 MS/MS scans) (Table S1). A total of 210 ion transitions was observed, of which 85 ion pairs were related to 36 Prec or NL scans for lipids and 125 ions were detected using 45 scans for metabolites. These Prec and NL scans have been previously reported and used in previous MRM profiling publications [5, 12, 13, 50]. Fig. 2 shows the Prec scan of *m/z* 184 used to profile phosphatidylcholine and sphingomyelin lipids, and the NL scan for 329 Da, which in the *m/z* range assayed, profiles for glycerolipids containing arachidic acid (C20:0).

For the MRM profiling screen, a large set of MRMs (N = 1357) for lipids and for metabolites (such as acylcarnitines) was also considered. This list combines isomers of the same class into one MRM. Therefore, these MRMs correspond to over 14,000 entries in the Lipid Maps Structure Database - LMSD (www.lipidmaps.org). These MRMs were associated with ceramides (Cer, N = 80), phosphatidylcholines and sphingomyelins (PC/SM, N = 147) phosphatidylethanolamines (PE, N = 151), phosphatidylglycerols (PG, N = 151), phosphatidylinositols (PI, N = 147), phosphatidylserines (PS, N = 147), cholesteryl esters (CE, N = 56), triacylglycerols (TAG, N = 383) and acyl-carnitines (AC, N = 59) as well as free fatty acids (FFA, N = 36). This set of MRMs was previously used for the analysis of bovine oocytes and embryos, as well as for mouse peri-ovarian fat as well as for swine milk and plasma [11,32,33]. Only the precursor ion mass was monitored for the free fatty acids since no informative fragmentation was observed with CID in the negative ion mode. Triacylglycerols (TAGs) were monitored using NL for C16:0 (NL 273 Da), C16:1 (NL 271 Da), C18:0 (NL 301 Da), C18:1 (NL 299 Da), C18:2 (NL 297 Da), C20:0 (NL 329 Da) and C20:4 (NL 321 Da) fatty acyl residues [34].

We also enriched the screening step by combining with the experimentally discovered MRMs, a list of 611 MRMs (363 in the positive mode, and 248 in the negative mode) related to 155 metabolites and related isomers (Table S3) based on the METLIN database and the literature [35,36]. Eventually 252 MRMs (190 in positive and 62 in the negative ion mode) related to 90 exogenous compounds (Table S4) of interest in environmental exposure and listed in the METLIN database and in the literature [15–30,37,38] were also combined with the discovery step MRM list. From all these MRMs, 191 MRMs related to lipids (Table S5), 104 MRMs related to 65 metabolites (Table S6), and 17 MRMS related to 14 exogenous compounds showed ion intensities above the threshold level (30% above the blank; Table S7). An overview of the number of detected MRMs in the screening step, which contained product ions related to the chemical functionality of lipids, metabolites, and exogenous compounds is depicted in Fig. 3.

In many applications of MRM profiling, the screening allows classification of samples into classes without the need to identify the individual compounds responsible for the classification. When this identification is required, the most straightforward way of obtaining it is to acquire product ion scan data for the precursors of the most intense MRMs. We did this for the 104 MRMs related to metabolites (Fig. S2) and for the 17 MRMs related to exogenous compounds (Fig. S3).

For the MRMs related to metabolites, fragments observed for 11 compounds allowed their tentatively identification as glycocholic acid (GCA), L-2-aminobutyric acid, glyceric acid, creatinine, L-proline, L-leucine/L-isoleucine, 4-hydroxy-L-proline, hypoxanthine, L-carnitine, α-D-glucose, D-galactose (Fig. S1). In the case of exogenous compounds, the observed fragments allowed just two tentative identifications (diethyl hexyl phthalate and acrylamide) (Fig. S2). These two compounds may be artifacts of the sample production as they are quickly metabolized and excreted and unlikely to be found in liver tissue. Monoesters of phthalate diesters were not screened for. The small fraction of compounds with assigned product ions in these experiments is due in part to the lower sensitivity of product ion scans compared to the MRM scan. Nonetheless, such experiments could help guide targeted LC-MS/MS or HRMS validation, steps out of the scope of this study and

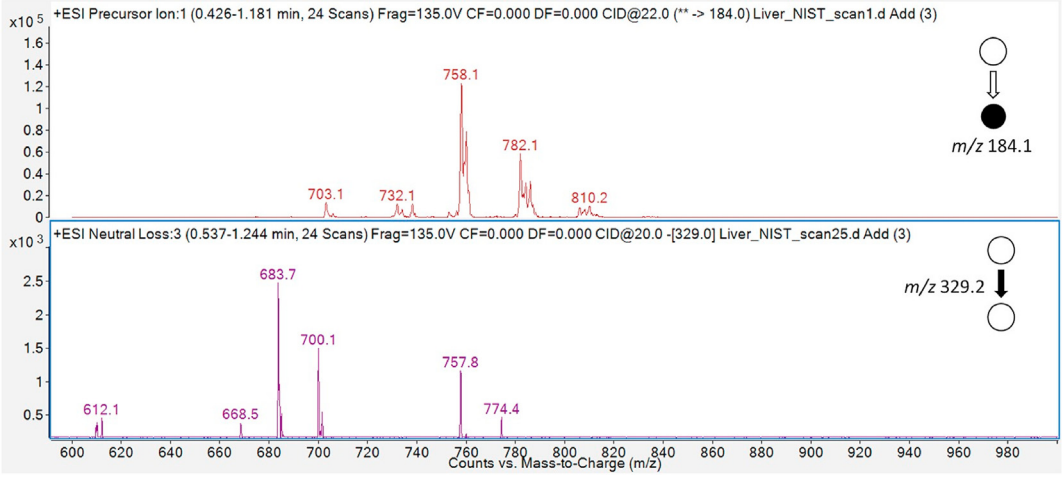

Fig. 2. Representative mass spectra of Prec and NL MS/MS scans applied to the NIST RGM 10122 sample: precursor ion (PC *m/z* 184) and neutral loss (glycerolipids containing arachidic acid residue NL of 329 Da). Masses labeled in the NL scan are those for the precursor ion.

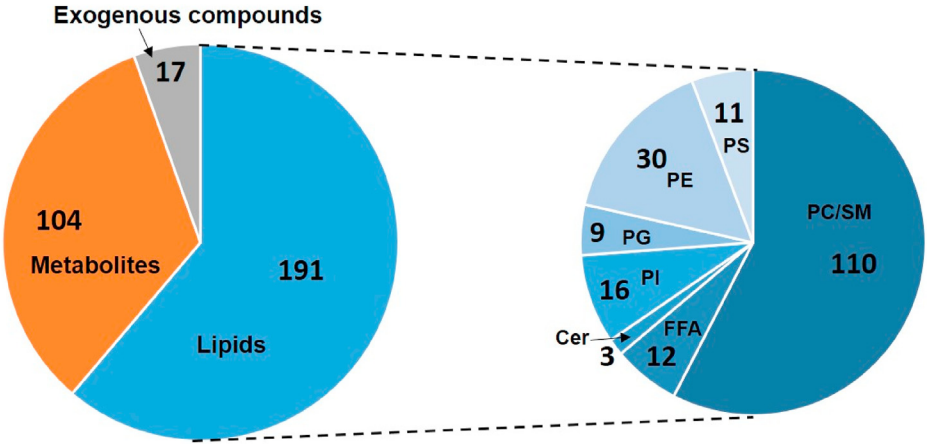

Fig. 3. Plot at left displays the number of transitions related to lipids, metabolites and exogenous compounds detected in the MRM profile screening step. The plot at right lists the number of MRMs for lipids by the lipid class.

the speed advantage of MRM profiling remains notable.

A reproducibility study of the MRM-profiling method was conducted using three different vials of the NIST RGM 10122 sample (Fig. S3). For the three sample vials, 237 MRMs were evaluated only based on total ion intensities (SM, Cer, AC, PI, FFA, PE, PS, PG, PC). The reproducibility results are summarized below in Fig. 4 and Table S8 where 79% of the measured lipids (188 out of 237 MRMs based on total ion intensities) showed % RSD lower than 25%. We did not observe a correlation between the ion abundance and the RSD for the classes of compounds profiled.

For the remaining 194 MRMs related to PC/SM, PG, PI, PS, and PE, normalization of data was performed using one IS per class (Fig. S5 and Table S9). The use of IS normalization for some lipid classes (SM, PI, PE, PS, PG, and PC) did not lower RSD values, most likely because the concentration of the IS used was not appropriate for all lipid classes monitored given the wide range of lipid concentrations. This suggestion is consistent with the fact that higher RSD values were observed for low abundance lipids (Tables S8 and S8). For the 58 MRMs related to metabolites studies for reproducibility (Table S10), average RSD was 12.3%. For the 11 MRMs related to exogenous compounds evaluated, average RSD observed was 13.5%.

For the entire reproducibility study, we used less than 1% of the sample (equivalent to 1 mg of tissue). Production of NIST RGM 10122 resulted in a known mass variance across vials as was exemplified by the results of vial number 2 which showed overall higher RSDs. Complimentary vials are available to the research community for feedback to help in the development of a system suitability sample Research Material (RM) or for use in laboratory method validation.

In summary, this exploratory analysis of the NIST RGM 10122 sample by the MRM profiling method used Prec, NL, and MRM scans to profile chemical classes, establishing MRM transitions for this important sample. The experiments were neither intended to be comprehensive nor specific to the sample type. The novelty of this research lies in the measurements on the NIST samples and the demonstration of reproducibility in the MRM-profiling study. The MRM profiling workflow is flexible, which means that exogenous compounds and known or predicted metabolites can be added to the screening methods. In the NIST RGM 10122 sample, MRM profiling allowed the detection of 191 MRMs related to lipids, 104 to

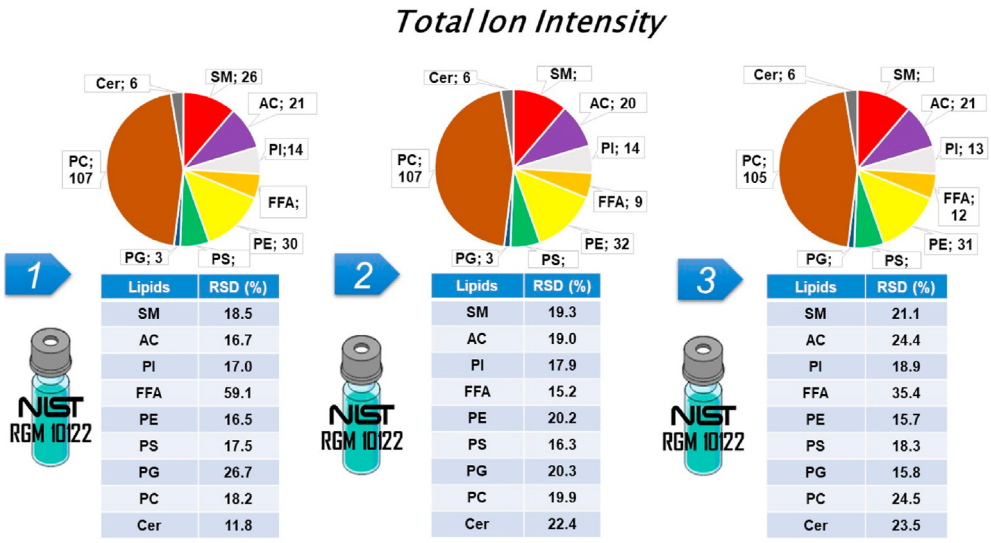

Fig. 4. Schematic showing %RSD for the data normalized by total ion intensities for each of the three RGM 10122 vials used in the MRM-profiling reproducibility analysis. The pie charts depict the number of lipids evaluated by lipid class. The MRMs that showed RSDs below 25% were 79% lipids (188 out of 237 MRMs based on total ion intensities). Comparisons across vials are not valid due to known variance during vial production.

metabolites, and 17 related to lipids, metabolites and exposome compounds, respectively. The % RSD was typically under 25% when using three different sample vials. The normalization of the data by addition of IS for some lipid classes did not lower the RSD values. As a fast and sensitive exploratory approach, this method can be used to guide the selection of molecules to be further validated by LC-MS/MS or HRMS.

Authors' contributions

MEE and CAM contributed equally to the experimental work and preparation of data for publication; TBS contributed systems knowledge and advice on presentation; TJPS made key contributions to data processing; CRF and RGC are responsible for conceiving the MRM profiling.

Disclaimer

Certain commercial equipment, instruments, or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We acknowledge the support of the National Science Foundation (NSF) CHE 1905087; Indiana Clinical and Translational Sciences Institute funded, in part, by Award Number UL1TR002529 from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences; Bindley Bioscience Center; and São Paulo Research Foundation (FAPESP n° 2019/03385-7 and n° 2018/11700-7). We appreciate the creativity, knowledge and effort of Clay Davis, Debra Ellisor, and the National Institute of Standards and Technology (NIST) Biorepository in the development of NIST RGM 10122.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijms.2021.116663.

References

[1] O. Quehenberger, A.M. Armando, A.H. Brown, S.B. Milne, D.S. Myers, A.H. Merrill, S. Bandyopadhyay, K.N. Jones, S. Kelly, R.L. Shaner, C.M. Sullards, E. Wang, R.C. Murphy, R.M. Barkley, T.J. Leiker, C.R.H. Raetz, Z. Guan, G.M. Laird, D.A. Six, D.W. Russell, J.G. McDonald, S. Subramaniam, E. Fahy, E.A. Dennis, Lipidomics reveals a remarkable diversity of lipids in human plasma1, J. Lipid Res. 51 (2010) 3299–3305, https://doi.org/10.1194/jlr.M009449.

[2] J.A. Bowden, A. Heckert, C.Z. Ulmer, C.M. Jones, J.P. Koelmel, L. Abdullah, L. Ahonen, Y. Alnouti, A.M. Armando, J.M. Asara, T. Bamba, J.R. Barr, J. Bergquist, C.H. Borchers, J. Brandsma, S.B. Breitkopf, T. Cajka, A. Cazenave-Gassiot, A. Checa, M.A. Cinel, R.A. Colas, S. Cremers, E.A. Dennis, J.E. Evans, A. Fauland, O. Fiehn, M.S. Gardner, T.J. Garrett, K.H. Gotlinger, J. Han, Y. Huang, A.H. Neo, T. Hyötyläinen, Y. Izumi, H. Jiang, H. Jiang, J. Jiang, M. Kachman, R. Kiyonami, K. Klavins, C. Klose, H.C. Köfeler, J. Kolmert, T. Koal, G. Koster, Z. Kuklenyik, I.J. Kurland, M. Leadley, K. Lin, K.R. Maddipati, D. McDougall, P.J. Meikle, N.A. Mellett, C. Monnin, M.A. Moseley, R. Nandakumar, M. Oresic, R. Patterson, D. Peake, J.S. Pierce, M. Post, A.D. Postle, R. Pugh, Y. Qiu, O. Quehenberger, P. Ramrup, J. Rees, B. Rembiesa, D. Reynaud, M.R. Roth, S. Sales, K. Schuhmann, M.L. Schwartzman, C.N. Serhan, A. Shevchenko, S.E. Somerville, L. St John-Williams, M.A. Surma, H. Takeda, R. Thakare, J.W. Thompson, F. Torta, A. Triebl, M. Trötzmüller, S.J.K. Ubhayasekera, D. Vuckovic, J.M. Weir, R. Welti, M.R. Wenk, C.E. Wheelock, L. Yao, M. Yuan, X.H. Zhao, S. Zhou, Harmonizing lipidomics: NIST interlaboratory comparison exercise for lipidomics using SRM 1950-Metabolites in frozen human plasma, J. Lipid Res. 58 (2017) 2275–2288, https://doi.org/10.1194/jlr.M079012.

[3] D.I. Walker, D. Valvi, N. Rothman, Q. Lan, G.W. Miller, D.P. Jones, The metabolome: a key measure for exposome research in epidemiology, Curr. Epidemiol. Rep. 6 (2019) 93–103, https://doi.org/10.1007/s40471-019-00187-4.

[4] J.C. Schwartz, A.P. Wade, C.G. Enke, R. Graham Cooks, Systematic delineation of scan modes in multidimensional mass spectrometry, Anal. Chem. 62 (1990) 1809–1818, https://doi.org/10.1021/ac00216a016.

[5] C.R. Ferreira, K.E. Yannell, B. Mollenhauer, R.D. Espy, F.B. Cordeiro, Z. Ouyang, R.G. Cooks, Chemical profiling of cerebrospinal fluid by multiple reaction monitoring mass spectrometry, Analyst 141 (2016) 5252–5255, https://doi.org/10.1039/c6an01618a.

[6] Z. Xie, C.R. Ferreira, A.A. Virequ, R.G. Cooks, Multiple reaction monitoring profiling (MRM profiling): small molecule exploratory analysis guided by chemical functionality, Chem. Phys. Lipids (2021) 105048, https://doi.org/10.1016/j.chemphyslip.2021.105048.

[7] P. Pastore, T. Rizzetto, O. Curcuruto, M.D. Cin, A. Zaramella, D. Marton, Characterization of dehydroascorbic acid solutions by liquid chromatography/mass spectrometry, Rapid Commun. Mass Spectrom. 15 (2001) 2051–2057, https://doi.org/10.1002/rcm.476.

[8] Z. Xie, L.E. Gonzalez, C.R. Ferreira, A. Vorsilak, D. Frabutt, T.J.P. Sobreira, M. Pugia, R.G. Cooks, Multiple reaction monitoring profiling (MRM-Profiling) of lipids to distinguish strain-level differences in microbial resistance in Escherichia coli, Anal. Chem. 91 (2019) 11349–11354, https://doi.org/10.1021/acs.analchem.9b02465.

[9] J. Franco, B. Rajwa, C.R. Ferreira, J.P. Sundberg, H. HogenEsch, Lipidomic profiling of the epidermis in a mouse model of dermatitis reveals sexual dimorphism and changes in lipid composition before the onset of clinical disease, Metabolites 10 (2020) 1–18, https://doi.org/10.3390/metabo10070299.

[10] L. Kobos, C.R. Ferreira, T.J.P. Sobreira, B. Rajwa, J. Shannahan, A novel experimental workflow to determine the impact of storage parameters on the mass spectrometric profiling and assessment of representative phosphatidylethanolamine lipids in mouse tissues, Anal. Bioanal. Chem. (2021), https://doi.org/10.1007/s00216-020-03151-0.

[11] S.S. Dipali, C.R. Ferreira, L.T. Zhou, M.T. Pritchard, F.E. Duncan, Histologic analysis and lipid profiling reveal reproductive age-associated changes in peri-ovarian adipose tissue, Reprod. Biol. Endocrinol. 17 (2019), https://doi.org/10.1186/s12958-019-0487-6.

[12] L.M. Kobos, S. Alqatani, C.R. Ferreira, U.K. Aryal, V. Hedrick, T.J.P. Sobreira, J.H. Shannahan, An integrative proteomic/lipidomic analysis of the gold nanoparticle biocorona in healthy and obese conditions, Appl. Vitr. Toxicol. 5 (2019) 150–166, https://doi.org/10.1089/aivt.2019.0005.

[13] D.J. Swiner, S. Jackson, B.J. Burris, A.K. Badu-Tawiah, Applications of mass spectrometry for clinical diagnostics: the influence of turnaround time, Anal. Chem. 92 (2020) 183–202, https://doi.org/10.1021/acs.analchem.9b04901.

[14] G.I.J. Salentijn, M. Grajewski, E. Verpoorte, Reinventing (Bio)chemical analysis with paper, Anal. Chem. 90 (2018) 13815–13825, https://doi.org/10.1021/acs.analchem.8b04825.

[15] Y. Sanchis, C. Coscollà, V. Yusà, Analysis of four parabens and bisphenols A, F, S in urine, using dilute and shoot and liquid chromatography coupled to mass spectrometry, Talanta 202 (2019) 42–50, https://doi.org/10.1016/j.talanta.2019.04.048.

[16] T. Fierens, K. Servaes, M. Van Holderbeke, L. Geerts, S. De Henauw, I. Sioen, G. Vanermen, Analysis of phthalates in food products and packaging materials sold on the Belgian market, Food Chem. Toxicol. 50 (2012) 2575–2583, https://doi.org/10.1016/j.fct.2012.04.029.

[17] Y. Li, T. Emm, S. Yeleswaram, Simultaneous determination of fluoxetine and its major active metabolite norfluoxetine in human plasma by LC-MS/MS using supported liquid extraction, Biomed. Chromatogr. 25 (2011) 1245–1251, https://doi.org/10.1002/bmc.1597.

[18] J. Jiang, D. Mu, M. Ding, S. Zhang, H. Zhang, J. Hu, Simultaneous determination of primary and secondary phthalate monoesters in the Taihu Lake: exploration of sources, Chemosphere 202 (2018) 17–24, https://doi.org/10.1016/j.chemosphere.2018.03.070.

[19] K.B. Scheidweiler, D.M. Shakleya, M.A. Huestis, Simultaneous quantification of nicotine, cotinine, trans-3'-hydroxycotinine, norcotinine and mecamylamine in human urine by liquid chromatography-tandem mass spectrometry, Clin. Chim. Acta 413 (2012) 978–984, https://doi.org/10.1016/j.cca.2012.02.017.

[20] Y. Peng, L. Gautam, S.W. Hall, The detection of drugs of abuse and pharmaceuticals in drinking water using solid-phase extraction and liquid chromatography-mass spectrometry, Chemosphere 223 (2019) 438–447, https://doi.org/10.1016/j.chemosphere.2019.02.040.

[21] I. Lee, R. Alakeel, S. Kim, Y.A. Al-Sheikh, H. Al-Mandeel, A.A. Alyousef, Y. Kho, K. Choi, Urinary phthalate metabolites among children in Saudi Arabia: occurrences, risks, and their association with oxidative stress markers, Sci. Total Environ. 654 (2019) 1350–1357, https://doi.org/10.1016/j.scitotenv.2018.11.025.

[22] G. Liebisch, M. Binder, R. Schifferer, T. Langmann, B. Schulz, G. Schmitz, High

throughput quantification of cholesterol and cholesteryl ester by electrospray ionization tandem mass spectrometry (ESI-MS/MS), Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1761 (2006) 121–128, https://doi.org/10.1016/j.bbalip.2005.12.007.

[23] R. Kumar, B. Tscharke, J. O'Brien, J.F. Mueller, C. Wilkins, L.P. Padhye, Assessment of drugs of abuse in a wastewater treatment plant with parallel secondary wastewater treatment train, Sci. Total Environ. 658 (2019) 947–957, https://doi.org/10.1016/j.scitotenv.2018.12.167.

[24] R.R. Taylor, K.L. Hoffman, B. Schniedewind, C. Clavijo, J.L. Galinkin, U. Christians, Comparison of the quantification of acetaminophen in plasma, cerebrospinal fluid and dried blood spots using high-performance liquid chromatography-tandem mass spectrometry, J. Pharmaceut. Biomed. Anal. 83 (2013) 1–9, https://doi.org/10.1016/j.jpba.2013.04.007.

[25] H.S. Cho, B. Cho, J. Sim, S.K. Baeck, S. In, E. Kim, Detection of 11-nor-9-carboxy-tetrahydrocannabinol in the hair of drug abusers by LC–MS/MS analysis, Forensic Sci. Int. 295 (2019) 219–225, https://doi.org/10.1016/j.forsciint.2018.12.013.

[26] A.J. Dunlop, I. Clunie, D.W.S. Stephen, J.J. Allison, Determination of cotinine by LC-MS-MS with automated solid-phase extraction, J. Chromatogr. Sci. 52 (2014) 351–356, https://doi.org/10.1093/chromsci/bmt038.

[27] V. García Ibarra, R. Sendón, J. Bustos, P. Paseiro Losada, A. Rodríguez Bernaldo de Quirós, Estimates of dietary exposure of Spanish population to packaging contaminants from cereal based foods contained in plastic materials, Food Chem. Toxicol. 128 (2019) 180–192, https://doi.org/10.1016/j.fct.2019.04.003.

[28] A.S. Ptolemy, E. Tzioumis, A. Thomke, S. Rifai, M. Kellogg, Quantification of theobromine and caffeine in saliva, plasma and urine via liquid chromatography-tandem mass spectrometry: a single analytical protocol applicable to cocoa intervention studies, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 878 (2010) 409–416, https://doi.org/10.1016/j.jchromb.2009.12.019.

[29] T. Gicquel, J. Aubert, S. Lepage, B. Fromenty, I. Morel, Quantitative analysis of acetaminophen and its primary metabolites in small plasma volumes by liquid chromatography-tandem mass spectrometry, J. Anal. Toxicol. 37 (2013) 110–116, https://doi.org/10.1093/jat/bks139.

[30] N.M. Morato, V. Pirro, P.W. Fedick, R.G. Cooks, Quantitative swab touch spray mass spectrometry for oral fluid drug testing, Anal. Chem. 91 (2019) 7450–7457, https://doi.org/10.1021/acs.analchem.9b01637.

[31] M. Höring, C.S. Ejsing, M. Hermansson, G. Liebisch, Quantification of cholesterol and cholesteryl ester by direct flow injection high-resolution fourier transform mass spectrometry utilizing species-specific response factors, Anal. Chem. 91 (2019) 3459–3466, https://doi.org/10.1021/acs.analchem.8b05013.

[32] C.B. de Lima, C.R. Ferreira, M.P. Milazzotto, T.J.P. Sobreira, A.A. Vireque, R.G. Cooks, Comprehensive lipid profiling of early stage oocytes and embryos by MRM profiling, J. Mass Spectrom. 53 (2018) 1247–1252, https://doi.org/10.1002/jms.4301.

[33] A. Suarez-Trujillo, K. Huff, C. Ramires Ferreira, T.J. Paschoal Sobreira, K.K. Buhman, T. Casey, High-fat-diet induced obesity increases the proportion of linoleic acyl residues in dam serum and milk and in suckling neonate circulation, Biol. Reprod. 103 (2020) 736–749, https://doi.org/10.1093/biolre/ioaa103.

[34] M. Li, E. Butka, X. Wang, Comprehensive quantification of triacylglycerols in soybean seeds by electrospray ionization mass spectrometry with multiple neutral loss scans, Sci. Rep. 4 (2014) 2251, https://doi.org/10.1038/srep06581.

[35] K.W. Phinney, G. Ballihaut, M. Bedner, B.S. Benford, J.E. Camara, S.J. Christopher, W.C. Davis, N.G. Dodder, G. Eppe, B.E. Lang, S.E. Long, M.S. Lowenthal, E.A. McGaw, K.E. Murphy, B.C. Nelson, J.L. Prendergast, J.L. Reiner, C.A. Rimmer, L.C. Sander, M.M. Schantz, K.E. Sharpless, L.T. Sniegoski, S.S.C. Tai, J.B. Thomas, T.W. Vetter, M.J. Welch, S.A. Wise, L.J. Wood, W.F. Guthrie, C.R. Hagwood, S.D. Leigh, J.H. Yen, N.F. Zhang, M. Chaudhary-Webb, H. Chen, Z. Fazili, D.J. Lavoie, L.F. McCoy, S.S. Momin, N. Paladugula, E.C. Pendergrast, C.M. Pfeiffer, C.D. Powers, D. Rabinowitz, M.E. Rybak, R.L. Schleicher, B.M.H. Toombs, M. Xu, M. Zhang, A.L. Castle, Development of a standard reference material for metabolomics research, Anal. Chem. 85 (2013) 11732–11738, https://doi.org/10.1021/ac402689t.

[36] K.H. Telu, X. Yan, W.E. Wallace, S.E. Stein, Y. Simón-Manso, Analysis of human plasma metabolites across different liquid chromatography/mass spectrometry platforms: cross-platform transferable chemical signatures (Rapid Commun. Mass Spectrom., (2016), 30, (581-593), 10.1002/rcm.7475), Rapid Commun. Mass Spectrom. 31 (2017) 152, https://doi.org/10.1002/rcm.7773.

[37] I. Ferrer, J.F. García-Reyes, M. Mezcua, E.M. Thurman, A.R. Fernández-Alba, Multi-residue pesticide analysis in fruits and vegetables by liquid chromatography-time-of-flight mass spectrometry, in: J. Chromatogr. A, Elsevier, 2005, pp. 81–90, https://doi.org/10.1016/j.chroma.2005.03.040.

[38] R.P. Lopes, Érica E. De Freitas Passos, J.F. De Alkimim Filho, E.A. Vargas, D.V. Augusti, R. Augusti, Development and validation of a method for the determination of sulfonamides in animal feed by modified QuEChERS and LC-MS/MS analysis, Food Contr. 28 (2012) 192–198, https://doi.org/10.1016/j.foodcont.2012.04.026.