

Cite this: *Analyst*, 2018, **143**, 1250

# Simultaneous non-polar and polar lipid analysis by on-line combination of HILIC, RP and high resolution MS†

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Given the chemical diversity of lipids and their biological relevance, suitable methods for lipid profiling and quantification are demanded to reduce sample complexity and analysis times. In this work, we present a novel on-line chromatographic method coupling hydrophilic interaction liquid chromatography (HILIC) dedicated to class-specific separation of polar lipid to reversed-phase chromatography (RP) for non-polar lipid analysis. More specifically, the void volume of the HILIC separation-consisting of non-polar lipids- is transferred to the orthogonal RP column enabling the on-line combination of HILIC with RP without any dilution in the second dimension. In this setup the orthogonal HILIC and RP separations were performed in parallel and the effluents of both columns were combined prior to high-resolution MS detection, offering the full separation space in one analytical run. Rapid separation for both polar and non-polar lipids within only 15 min (including reequilibration time) was enabled using sub-2  $\mu\text{m}$  particles and UHPLC. The method proved to be robust with excellent retention time stability (RSDs < 1%) and LODs in the fmol to pmol (absolute on column) range even in the presence of complex biological matrix such as human plasma. The presented high-resolution LC-MS/MS method leads to class-specific separation of polar lipids and separation of non-polar lipids which is lost in conventional HILIC separations. HILIC-RP-MS is a promising tool for targeted and untargeted lipidomics workflows as three interesting features are combined namely (1) the decreased run time of state of the art shotgun MS methods, (2) the elevated linear dynamic range inherent to chromatographic separation and (3) increased level of identification by separation of polar and non-polar lipid classes.

Received 8th December 2017,  
Accepted 5th February 2018

DOI: 10.1039/c7an01984j

rsc.li/analyst

## 1. Introduction

Lipids can be classified into categories by their chemical and structural similarity,<sup>1</sup> they can be grouped into polar and non-polar lipids based on their overall hydrophobicity or categorized by their molecular building blocks.<sup>2</sup> Given the extremely high diversity of lipids (over 40 000 unique lipid structures annotated in the Lipid Maps Structure Database<sup>3,4</sup>) and increasing proof of their biological relevance,<sup>3,5–9</sup> the urge to develop novel methods for lipid profiling and quantification continues with the major aim to reduce sample complexity

and analysis times. High-resolution mass spectrometry (HRMS) has evolved as a key technique in lipidomics as it provides lipid identification by accurate mass and fragmentation pattern at the same time allowing to cope with complex samples.<sup>10</sup> On general terms, MS based lipidomics strategies involve (1) direct-infusion shotgun lipidomics approaches<sup>10–12</sup> and/or (2) the combination of liquid chromatography (LC) and MS.<sup>2,13–18</sup> Shotgun lipidomics offers the advantage of fast lipid profiling but the direct infusion leads to problems with isomeric and isobaric lipid species and a limited dynamic range in a sample of interest. LC-MS based approaches offer (1) an increased dynamic range and (2) an additional level of identification by retention time. Different chromatographic separations were developed for lipidomics tasks including reversed-phase chromatography (RP), normal phase chromatography (NP), hydrophilic interaction chromatography (HILIC), strong anion exchange chromatography (SAX) and supercritical fluid chromatography (SFC).<sup>19–25</sup> Indeed, RP chromatography separates lipids based on hydrophobic properties such as fatty acid chain length, degree of saturation and double bond position.

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†Electronic supplementary information (ESI) available. See DOI: 10.1039/c7an01984j





**Fig. 1** Setup for HILIC-RP-MS. A two position six-port valve is used to transfer the void volume from the first dimension (HILIC) to the second dimension (RP). The valve was set to position A from 0–1.5 min, to position B from 1.5–14.1 min and for equilibration reasons prior to the next run again to position A from 14.1–15.0 min. A. Serial configuration: The HILIC column was directly connected with the RP column to transfer the void volume. B. Parallel configuration: The HILIC and the RP column used different eluent systems by two separate UHPLC pumps. Pump and MS icons were created via the Mind the Graph platform.<sup>56</sup>

HILIC column were transferred to the second RP dimension. At 1.5 min the valve was switched to position B (Fig. 1) directing the second-dimension pump flow to the RP column. The column effluents were combined using a T-piece prior to the introduction into the ESI source of the mass spectrometer.

## 2.6 High-resolution mass spectrometry (HRMS)

High-resolution MS with a Q Exactive HF (Thermo Fisher Scientific) was used for lipid detection. The following HESI source parameters were applied: capillary temperature of 270 °C, sheath gas flow rate of 50, auxiliary flow rate of 14, sweep gas of 3, S-lens RF level of 45 and auxiliary gas heater temperature of 380 °C applying a spray voltage of 3.5 kV in positive mode and 2.8 kV in negative mode. Full-MS mode at 120 000 resolution with an AGC target of 1e6 was used for the quantification runs. A top 10 ddMS2 method with inclusion list (using generated<sup>57</sup> suspect lists for human plasma and yeast samples deduced from literature with assigned retention times determined by standards) was applied for the identification runs using 60 000 MS1 resolution and 15 000 MS2 resolution as well as normalized collision energies of 25 (+) and 28 (–). Spectral data was recorded in the mass range of 200–2000 *m/z* using profile mode. All triggered masses were set on the exclusion list for 15 s and if no masses of the inclusion list were found, ddMS2 spectra were recorded. Human plasma and yeast were also analyzed by direct infusion shotgun analysis using the robotic nanoflow ion source TriVersa NanoMate (Advion BioSciences, Ithaca NY, USA) into a Q Exactive HF

instrument (Thermo Fisher Scientific, Bremen, Germany) followed by LipidXplorer analysis.<sup>11,58</sup> Additional details are provided in the extended method section of the ESI.†

## 2.7 Data evaluation of HILIC-RP-HRMS

Data evaluation was performed using Lipid Search 4.1 (Thermo Fisher Scientific) for the ddMS2 identification runs (*n* = 3 samples and one sample was measured twice as analytical replicate, measurement in positive and negative mode). Lipid Search results were filtered for 5 ppm in MS1, 7 ppm in MS2 and the lipids were only considered if the areas were 3 × higher than in the blank samples or not present in the blanks at all. The main adduct ions in positive mode was set to H<sup>+</sup> for PC, PS, PE, PA, HexCer, SM, AcCa, for MG, DG, TG, PG, PI, CE the main adduct ions were set to M + NH<sub>4</sub>/Na, for Cer, and HexCer additionally adduct ion with loss of H<sub>2</sub>O were considered. The main adduct ions in negative mode was set to H<sup>–</sup> for PS, PE, PA, Cer, HexCer, SM, AcCa, for PC and SM the main adduct ion was set to HCOO<sup>–</sup>, for Cer, and HexCer additionally adduct ion with loss of H<sub>2</sub>O were considered. The main grade was set to A (lipid class and fatty acids are completely identified) and B (lipid class and some fatty acids are identified) for all lipid classes except PC, Cer, HexCer and SM, there A, B and C (lipid class or fatty acids are identified) grade were allowed. Tracefinder 4.1 (Thermo Fisher Scientific) was used for Full-MS quantification of lipid standards based on peak areas obtained from extracted ion chromatograms (±5 ppm) with external calibration. The calibration was performed over four

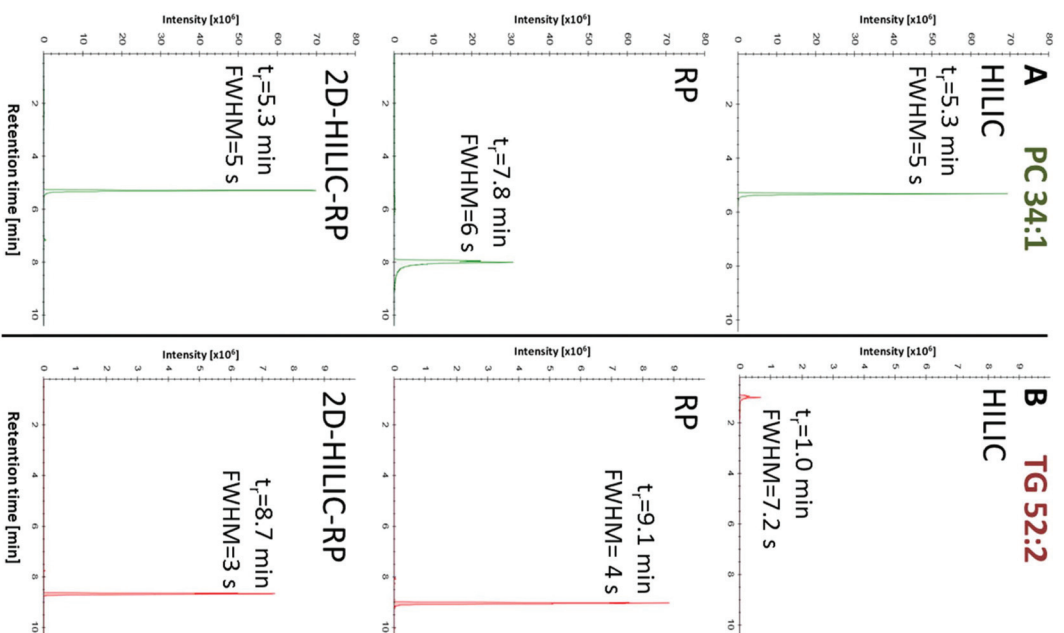


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**Table 1** Separation and quantification of 20 lipids originating from 15 lipid classes using RP-HILIC-HRMS (+/–) in human plasma ( $n = 3$ ) and yeast ( $n = 3$ ). Information on lipid short notation, MS polarity mode, retention time, linearity ( $R^2$ ), limit of detection (LOD), adduct formation, and  $m/z$  can be found as well as lipid concentrations determined in the samples can be found

| Lipid class                   | Standards                  | Sum formula | MS  | RT (min) | Linearity ( $R^2$ ) | LOD (nM) | Adduct              | $m/z$ (+) | $m/z$ (–) | SRM ( $n = 3$ )<br>nmol mL <sup>–1</sup> |       | Yeast ( $n = 3$ )<br>μmol 10 <sup>7</sup> per<br>cells |       |
|-------------------------------|----------------------------|-------------|-----|----------|---------------------|----------|---------------------|-----------|-----------|--|-------|--|-------|
|                               |                            |             |     |          |                     |          |                     |           |           | Average                                  | SD    | Average  | SD    |
| Ceramide (Cer)                | Cer d36:1                  | C36H71NO3   | +   | 7.88     | 0.9952              | 14       | M + H               | 566.551   |           | 0.18                                     | 0.01  | 0.011  | 0.004 |
| Cholesteryl ester (CE)        | CE 18:0                    | C45H80O2    | +   | 9.65     | 0.9842              | 83       | M + NH <sub>4</sub> | 670.650   |           | 8.21                                     | 3.07  | <LOD   |       |
| Cholesteryl ester (CE)        | CE 18:2                    | C45H76O2    | +   | 9.47     | 0.9959              | 83       | M + NH <sub>4</sub> | 666.618   |           | 73.33                                    | 18.93 | 0.006  | 0.001 |
| Diacylglycerol (DG)           | DG 34:1                    | C37H70O5    | +   | 7.95     | 0.9933              | 5        | M + NH <sub>4</sub> | 617.512   |           | 8.90                                     | 0.66  | 0.651  | 0.085 |
| Fatty acid (FA)               | FA 16:0                    | C16H32O2    | –   | 7.48     | 0.9981              | 2        | M – H               |           | 255.233   | 64.43                                    | 3.52  | 4.493  | 0.662 |
| Fatty acid (FA)               | FA 18:0                    | C18H36O2    | –   | 7.64     | 0.9922              | 1        | M – H               |           | 283.265   | 9.58                                     | 0.05  | 4.237  | 0.836 |
| Hexosyl ceramide (HexCer)     | HexCer d34:1               | C40H77NO8   | +   | 3.01     | 0.9967              | 5        | M + H               | 700.572   |           | <LOD                                     |       | <LOD   |       |
| Dihexosylceramide (Hex2Cer)   | Hex <sub>2</sub> Cer d34:1 | C46H87NO13  | +   | 5.88     | 0.9970              | 59       | M + H               | 862.625   |           | <LOD                                     |       | <LOD   |       |
| Lysophosphatidylcholine (LPC) | LPC 16:0                   | C24H50NO7P  | +/- | 6.1      | 0.9962              | 2        | M + H/M + HCOO      | 496.340   | 540.331   | 39.71                                    | 2.24  | 0.052  | 0.007 |
| Lysophosphatidylcholine (LPC) | LPC 18:0                   | C26H54NO7P  | +/- | 6.03     | 0.9965              | 1        | M + H/M + HCOO      | 524.371   | 568.362   | 23.93                                    | 2.13  | 0.029  | 0.004 |
| Phosphatidic acid (PA)        | PA 36:2                    | C39H73O8P   | –   | 5.62     | 0.9933              | 1015     | M – H               |           |           | 699.497                                  |       | 19.616   | 3.272 |
| Phosphatidylcholine (PC)      | PC 34:1                    | C42H82NO8P  | +/- | 5.3      | 0.9921              | 1        | M + H/M + HCOO      | 760.585   | 804.576   | 65.09                                    | 3.99  | 2.501  | 0.434 |
| Phosphatidylcholine (PC)      | PC 34:2                    | C42H80NO8P  | +/- | 5.31     | 0.9916              | 1        | M + H/M + HCOO      | 758.569   | 802.560   | 122.62                                   | 7.02  | 10.678   | 1.558 |
| Phosphatidylcholine (PC)      | PC O-36:2                  | C44H86NO7P  | +   | 5.23     | 0.9965              | 1        | M + H/M + Cl        | 772.621   | 806.584   | 0.78                                     | 0.60  | 4.139  | 0.841 |
| Phosphatidylethanolamine (PE) | PE 36:2                    | C41H78NO8P  | +/- | 5.31     | 0.9940              | 4        | M + H/M – H         | 744.554   | 742.539   | 5.15                                     | 0.16  | 39.125   | 6.291 |
| Phosphatidylglycerol (PG)     | PG 36:2                    | C42H79O10P  | +/- | 4.26     | 0.9955              | 76       | M + H/M – H         | 775.548   | 773.534   | <LOD                                     |       | 0.078  | 0.009 |
| Phosphatidylserine (PS)       | PS 36:2                    | C42H78NO10P | –   | 6.19     | 0.9889              | 640      | M – H               |           | 786.529   | <LOD                                     |       | 3.310  | 0.790 |
| Sphingomyelin (SM)            | SM d42:2                   | C47H93N2O6P | +   | 5.71     | 0.9858              | 6        | M + H               | 813.684   |           | 10.20                                    | 0.83  | <LOD   |       |
| Sterol (ST)                   | ST 28:3                    | C28H44O     | +   | 7.85     | 0.9568              | 104      | M + H               | 397.347   |           | <LOD                                     |       | 2.981  | 0.403 |
| Triacylglycerol (TG)          | TG 52:2                    | C55H102O6   | +   | 8.67     | 0.9931              | 4        | M + NH <sub>4</sub> | 881.757   |           | 7.10                                     | 1.36  | 0.564  | 0.047 |



**Fig. 3** Comparison of peak widths using HILIC and RP separately or coupled (HILIC-RP), shown exemplarily for the extracted ion chromatograms of PC 34:1 (A) and TG 52:2 (B) detected by HRMS in positive mode. Retention time ( $t_r$ ) is given in minutes and the peak width is calculated via full peak width at half-maximal peak height (FWHM). The chromatogram shows that the non-polar lipids were successfully loaded onto the RP column while the comparison between HILIC and HILIC-RP peak widths shows that the on-line coupling does not introduce peak broadening.

### 3.2 HILIC-RP-HRMS applications in lipidomics

After successful method development, the final HILIC-RP-MS method was applied to characterize two different sample types namely *Pichia pastoris* yeast and human serum (SRM 1950) samples. Lipids were extracted accordingly by Folch extraction<sup>53</sup> for yeast samples ( $n = 3$ ) and MTBE extraction for human plasma samples ( $n = 3$ ).<sup>54</sup> On-line HILIC-RP chromatography was coupled to a high-resolution mass spectrometer (Q Exactive HF).

### 3.3 Untargeted screening by HILIC-RP-HRMS

A panel of 20 lipid standards covering 14 lipid classes (CE, DG, HexCer, Hex<sub>2</sub>Cer, FA, LPC, PA, PC, PE, PG, PS, SM, ST, TG) was



**Assessment of HILIC-RP-HRMS quantification capabilities.** Finally, the novel HILIC-RP-HRMS approach was investigated regarding the analytical figures of merit of retention time stability, linear dynamic range and limit of detection using a panel of 20 lipids (Table 1). Linear calibration curves over 4

orders of magnitude could be obtained for most standards (0.01–10  $\mu\text{M}$ ) (Table 1, ESI Fig. S3<sup>†</sup>). LODs were calculated using the lowest detected concentration point ( $n = 3$ ) of the calibration<sup>61</sup> and were found in the low to high nM range (corresponding to low fmol to low pmol absolute on column) depending on the lipid species analyzed (Table 1) as reported for other LC-MS based lipidomics approaches.<sup>62,63</sup> Compared to classical shotgun based workflows, the method was superior, as previous results reported LODs and LOQs in the low to high  $\mu\text{M}$  range for the target lipids.<sup>64</sup> Both the retention time and the peak area precision were excellent with RSDs  $\leq 1\%$  and  $<5\%$  respectively (over a measurement period of 44 h) for all lipids monitored. In a next step, preliminary quantification of 20 target lipid was performed by external calibration in SRM 1950 human plasma ( $n = 3$ ) and *Pichia pastoris* yeast ( $n = 3$ ) samples. Lipid concentrations determined in the latter sample type were in the nmol to  $\mu\text{mol}$  range for  $10^7$  cells (Table 1) and compared well to previous studies on *Pichia pastoris* or other yeasts.<sup>34,65–68</sup> Trueness bias of analysis was assessed in the SRM 1950 using the recently introduced accuracy assessment tool LipidQC,<sup>69</sup> which followed the publication of a NIST interlaboratory comparison report on high molecular lipids<sup>70,71</sup> (ESI Fig. S4<sup>†</sup>). After normalization by volume the obtained values proved to be in agreement with the reported values for several lipid classes as all quantified lipids were in the nmol  $\text{mL}^{-1}$  range. It has to be noted however, that accurate absolute quantification strategies in lipidomics involve internal standardization. External calibration without internal standardization as carried out in the proof of principle experiments here is not recommended for lipids retained on reversed phase separations. Despite this fact, the preliminary data on the reference material exemplified the potential of the HILIC-RP-HRMS method for future quantification studies (involving internal standards). As can be seen in ESI Fig. S4,<sup>†</sup> the accuracy assessment revealed that 13 out of 15 lipid standards (FA 16:0 DG 34:1, TG 52:2, LPC 16:0, LPC 18:0, PC 34:1, PC 34:2, PC O-36:2, PE 36:2, Cer d36:1, SM d42:2, CE 18:0, CE 18:2) fell within the 99% confidence interval of the consensus mean value,<sup>69</sup> despite the fact that only external calibration was implemented not compensating for matrix effects and losses during sample preparation. This preliminary data show the potential of the HILIC-RP-HRMS method. Future studies will focus on implementation of a routine quantification method establishing internal standards and automatic deisotoping algorithm. Overall, the results show that fast in-depth profiling and quantification is possible by HILIC-RP-HRMS with increased level of lipid identification by retention time information. The class-specific separation on the HILIC column allows class-specific lipid quantification in 15 min as performed in state of the art shotgun lipidomics approaches.<sup>10,72,73</sup> Moreover, the on-line coupled RP column enables additional non-polar lipid trapping compared to conventional HILIC methods.<sup>28</sup> Finally, co-elution of isobars from different lipid classes is avoided and the linear dynamic range is increased compared to shotgun lipidomics approaches. Hence, HILIC-RP-MS is able to combine several interesting fea-

tures (1) the decreased run time of state of the art shotgun MS methods, (2) the elevated linear dynamic range inherent to chromatographic separation and (3) increased level of identification by separation of polar and non-polar lipid classes.

## 4. Conclusion

With the advent of sub-2  $\mu\text{m}$  particles and UHPLC new chromatographic possibilities became available.<sup>37–41</sup> In this work, we show the power of on-line combinations of sub-2  $\mu\text{m}$  HILIC and RP coupled to HRMS for lipid analysis. By addition of a 6-port valve and a T-piece, direct coupling of the HILIC column to the RP column was possible for simultaneous analysis of polar and non-polar lipids. This setup enables on-line combination of HILIC with RP without any dilution (or use of a trapping column) in the second dimension, exploiting the strong interaction of hydrophobic lipids and the enhanced separation space offered by the orthogonal methods. The fast chromatographic run time of 15 min is comparable to state of the art direct-infusion shotgun lipidomics profiling approaches.<sup>10–12</sup> These results show that HILIC-RP-HRMS is a valuable tool for high-throughput lipidomics analysis, bridging the gap between state of the art shotgun and LC-MS approaches. Moreover, we strongly believe that lipidomics studies will benefit from the increased separation space, enhanced sample throughput and broader lipid information of on-line coupled HILIC-RP-MS methods.

## Abbreviations

|                      |  |
|----------------------|--|
| ST                   | Sterols                                |
| GPL                  | Glycerophospholipids                   |
| DG                   | Diglyceride                            |
| TG                   | Triglyceride                           |
| MG                   | Monoglyceride                          |
| Cer                  | Ceramide                               |
| CE                   | Cholesterol ester                      |
| PE                   | Phosphatidylethanolamine               |
| LPE                  | Lysophosphatidylethanolamine           |
| DMPE                 | Dimethyl-phosphatidylethanolamine      |
| PC                   | Phosphatidylcholine                    |
| LPC                  | Lysophosphatidylcholine                |
| PG                   | Phosphatidylglycerol                   |
| PI                   | Phosphatidylinositol                   |
| PS                   | Phosphatidylserine                     |
| PA                   | Phosphatidic acid                      |
| SM                   | Sphingomyelin                          |
| FA                   | Fatty acids                            |
| AcCa                 | Acyl carnitine                         |
| HexCer               | Hexosyl ceramide                       |
| Hex <sub>2</sub> Cer | Dihexosyl ceramide                     |
| Co                   | Coenzyme                               |
| HRMS                 | High resolution mass spectrometry      |
| HILIC                | Hydrophilic interaction chromatography |

|       |  |
|-------|--|
| RP    | Reversed-phase chromatography                |
| UHPLC | Ultra-high performance liquid chromatography |

## Conflicts of interest

The authors declare no competing financial interest.

## Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version.

## Acknowledgements

This work was supported by the University of Vienna, the Faculty of Chemistry, the Vienna Metabolomics Center (VIME; <http://metabolomics.univie.ac.at/>) and the research platform Chemistry Meets Microbiology of the University of Vienna. The authors thank all members of the Environmental Analysis (University of Vienna) group for continuous support. Especially, we acknowledge Petra Volejnik and Sophie Neumayer for laboratory support. Moreover, the collaborative work with Gerrit Hermann (ISotopic Solutions, Vienna) is highly appreciated.

## References

- 1 E. Fahy, *J. Lipid Res.*, 2005, **46**, 839–862.
- 2 X. Han, *Lipidomics: Comprehensive mass spectrometry of lipids*, John Wiley & Sons, Hoboken, New Jersey, 2016.
- 3 M. Sud, E. Fahy, D. Cotter, A. Brown, E. A. Dennis, C. K. Glass, A. H. Merrill, R. C. Murphy, C. R. H. Raetz, D. W. Russell and S. Subramaniam, *Nucleic Acids Res.*, 2007, **35**, 527–532.
- 4 <http://www.lipidmaps.org>.
- 5 E. Fahy, S. Subramaniam, R. C. Murphy, M. Nishijima, C. R. H. Raetz, T. Shimizu, F. Spener, G. van Meer, M. J. O. Wakelam and E. a. Dennis, *J. Lipid Res.*, 2009, **50**(Suppl), S9–S14.
- 6 G. van Meer, D. R. Voelker and G. W. Feigenson, *Nat. Rev. Mol. Cell Biol.*, 2008, **9**, 112–124.
- 7 S. Daemen, M. A. M. J. van Zandvoort, S. H. Parekh and M. K. C. Hesselink, *Mol. Metab.*, 2016, **5**, 153–163.
- 8 S. Martin and R. G. Parton, *Semin. Cell Dev. Biol.*, 2005, **16**, 163–174.
- 9 A. Shevchenko and K. Simons, *Nat. Rev. Mol. Cell Biol.*, 2010, **11**, 593–598.
- 10 D. Schwudke, K. Schuhmann, R. Herzog, S. Bornstein and A. Shevchenko, *Cold Spring Harbor Perspect. Biol.*, 2011, **3**, 1–13.





- 60 K. Sandra, A. dos S. Pereira, G. Vanhoenacker, F. David and P. Sandra, *J. Chromatogr. A*, 2010, **1217**, 4087–4099.
- 61 B. Magnusson and U. Örnemark, *Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics*, 2nd edn, 2014.
- 62 X. Liu, Z. Ser and J. W. Locasale, *Anal. Chem.*, 2014, **86**, 2175–2184.
- 63 J. Zhou, H. Liu, Y. Liu, J. Liu, X. Zhao and Y. Yin, *Anal. Chem.*, 2016, **88**, 4478–4486.
- 64 M. A. Surma, R. Herzog, A. Vasilj, C. Klose, N. Christinat, D. Morin-Rivron, K. Simons, M. Masoodi and J. L. Sampaio, *Eur. J. Lipid Sci. Technol.*, 2015, **117**, 1540–1549.
- 65 C. S. Ejsing, J. L. Sampaio, V. Surendranath, E. Duchoslav, K. Ekroos, R. W. Klemm, K. Simons and A. Shevchenko, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 2136–2141.
- 66 L. Klug, P. Tarazona, C. Gruber, K. Grillitsch, B. Gasser, M. Trötzlmüller, H. Köfeler, E. Leitner, I. Feussner, D. Mattanovich, F. Altmann and G. Daum, *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids*, 2014, **1841**, 215–226.
- 67 V. A. Ivashov, K. Grillitsch, H. Köfeler, E. Leitner, D. Bäumlisberger, M. Karas and G. Daum, *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids*, 2013, **1831**, 282–290.
- 68 K. Grillitsch, P. Tarazona, L. Klug, T. Wriessnegger, G. Zellnig, E. Leitner, I. Feussner and G. Daum, *Biochim. Biophys. Acta*, 2014, **1838**, 1889–1897.
- 69 C. Z. Ulmer, J. M. Ragland, J. P. Koelmel, A. Heckert, C. M. Jones, T. Garrett, R. A. Yost and J. A. Bowden, *Anal. Chem.*, 2017, **89**(24), 13069–13073.
- 70 J. A. Bowden, C. Z. Ulmer, C. M. Jones and A. Heckert, *National Institute of Standards and Technology-Interagency/ Internal Report (NISTIR)*, 2017, vol. 8185, pp. 1–451.
- 71 J. A. Bowden, A. Heckert, C. Z. Ulmer, C. M. Jones, J. P. Koelmel, L. Abdullah, L. Ahonen, Y. Alnouti, A. 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. Huipeng Neo, T. Hyötyläinen, Y. Izumi, H. Jiang, H. Jiang, J. Jiang, M. Kachmann, 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, M. Danielle, 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. Sommerville, L. St. John-Williams, M. A. Surma, H. Takeda, R. Thakare, J. W. Thompson, F. Torta, A. Triebel, M. Trötzlmüller, K. Ubhayasekera, D. Vuckovic, J. M. Weir, R. Welti, M. R. Wenk, C. E. Wheelock, L. Yao, M. Yuan, X. H. Zhao and S. Zhou, *J. Lipid Res.*, 2017, **58**(12), 2275–2288.
- 72 C. S. Ejsing, J. L. Sampaio, V. Surendranath, E. Duchoslav, K. Ekroos, R. W. Klemm, K. Simons and A. Shevchenko, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 2136–2141.
- 73 K. Yang and X. Han, *Metabolites*, 2011, **1**, 21–40.

