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REVIEW

Applications of Multiple Reaction Monitoring to Clinical Glycomics

L. Renee Ruhaak · Carlito B. Lebrilla

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Abstract Multiple reaction monitoring or MRM is widely acknowledged for its accuracy of quantitation. The applications have mostly been in the analysis of small molecules and proteins, but its utility is expanding. Protein glycosylation was recently identified as a new paradigm in biomarker discovery for health and disease. A number of recent studies have now identified differential glycosylation patterns associated with health and disease states, including aging, pregnancy, rheumatoid arthritis, and different types of cancer. While the use of MRM in clinical glycomics is still in its infancy, it can likely play a role in the quantitation of protein glycosylation in the clinical setting. Here, we aim to review the current advances in the nascent application of MRM in the field of glycomics.

Keywords Multiple reaction monitoring · Glycomics · Mass spectrometry · Biofluids

Introduction

As one of the most common post-translational modifications of proteins, present in at least 70 % of plasma and membrane proteins, glycosylation is involved in many key biological processes. The glycan moiety of glycoproteins plays a role in protein folding [1] and protein stability [2] as well as cell–cell interactions, cell–matrix interactions [3] and receptor binding and activation. Given the importance of these processes in human health, it is not surprising that differential glycosylation has been observed in numerous disease states including several types of cancer [4–8], autoimmune diseases [9, 10], and diabetes [11]. Moreover, altered glycosylation profiles have been observed with aging [12, 13] and during pregnancy [14, 15]. Analytical methods suitable for fast and robust profiling of glycosylation—preferably at the level of individual proteins—are instrumental to further advance the use of glycomics in a clinical setting. Multiple reaction monitoring (MRM) has the potential to be one of the key techniques in this field.

The ability to quantitate accurately is an important aspect of clinical mass spectrometry. For many years, MRM has been the standard for quantitation in targeted applications, specifically in proteomics [16] and metabolomics [17]. MRM is commonly performed on triple quadrupole (QQQ) instruments and is widely acknowledged for its quantitative properties. In MRM with a QQQ, the first and third quadrupole are used as mass-selective filters that specifically select the analyte of interest (Q1) and a characteristic fragment ion of the analyte (Q3). The second quadrupole (Q2) functions as a collision cell with a broad m/z passage range, where the analyte is fragmented under collision-induced dissociation (CID) conditions. A pair of analyte/fragment ions is typically referred to as a transition. Multiple transitions are detected in a single run as co-eluting background analytes (often originating from sample impurities) are filtered out in the two stages of selection resulting in high selectivity of the method. The targeted nature of the analysis provides high sensitivity and linearity

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L. R. Ruhaak (✉) · C. B. Lebrilla
Department of Chemistry, University of California Davis, One Shields Avenue, Davis, CA 95616, USA
e-mail: lrruhaak@gmail.com

Present Address:
L. R. Ruhaak
Department of Translational Molecular Pathology, MD Anderson Cancer Center, 6767 Bertner Avenue, Houston, TX 77030, USA

over a wide dynamic range. Therefore, MRM methods are very suitable for the robust, fast, sensitive, and specific quantitative analysis of multiple compounds simultaneously in the presence of other more abundant compounds (e.g., in complicated mixtures, such as biofluids).

Because MRM is a targeted approach, knowledge regarding the mass and charge state of the analyte as well as its fragmentation behavior in CID is essential for the development of the transitions. Several studies have focused on the fragmentation pattern of glycans and glycopeptides [18–20]. In low energy CID conditions found in standard QQQ instruments, the typical fragmentation occurs at the glycosidic bonds. This allows for the determination of monosaccharide connectivity, but in this mode there is usually little that distinguishes the different linkage positions. Under low energy conditions of native compounds cross-ring cleavages are often of low abundances. Therefore, CID of N-glycans and N-glycopeptides yields primarily glycan oxonium ions at higher abundances resulting from the cleavage of glycosidic bonds.

Recent advances have been made in the use of MRM in the field of glycobiology and glycomics; however, its use and application in biological studies is still in its infancy and rather limited. This review aims to provide an overview of the nascent utility of MRM for the analysis of glycans and glycoconjugates with an emphasis on its application in the clinical setting. It is structured based on the different types of analytes: quantification of glycoproteins, quantification of glycosylation at the glycopeptide level, and quantification of glycans.

Quantification of Glycoproteins

One of the more commonly applied strategies is the quantitation of glycosylated proteins using MRM. Methods for glycoprotein or glycopeptide analysis involved extensive enrichment of target analytes from a complicated biological sample for subsequent quantitation. Several methods for enrichment have been employed either based on global glycan enrichment (e.g., [21]) or based on the enrichment of specific glycan features using lectins (e.g., [22]).

A detailed protocol for a global enrichment strategy using glycan oxidation and hydrazine-based enrichment of glycoproteins (solid-phase extraction of N-linked glycoprotein/peptide (SPEG)) has been described [23]. The proteins were first immobilized at the glycan moiety and then tryptic digested. PNGase F was then used to release the immobilized glycan from the peptide moiety, resulting in free deglycosylated peptides. Monitoring the deglycosylated peptide was performed with synthetic and isotopically labeled peptides used for both MRM optimization and quantitation of the glycoprotein [23]. While no immediate application of the method was described, an adaptation of the method was used

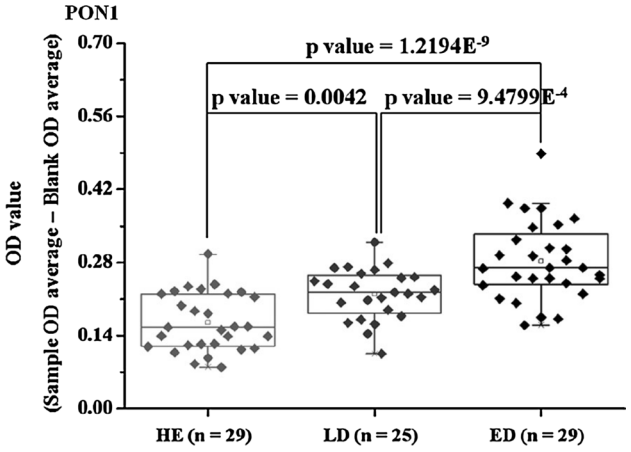

Fig. 1 Differential levels of fucosylated PON1 in the serum of small cell lung cancer patients. Patients had either limited disease (LD) or extended disease (ED) and were compared to healthy controls (HE). Fucosylated proteins were enriched using the AAL lectin, followed by tryptic digestion and MRM-based protein quantification. Significant results (including PON1) were then validated using hybrid AAL ELISA, of which the intensities are represented. The *P* values were calculated using non-parametric Kruskall–Wallis analysis of variance. Reprinted from [28]

to evaluate the glycosylation of prostate-specific antigen (PSA) in prostate cancer and non-cancer tissues [24]. Here, both the global SPEG method, as well as a sialic acid-specific method using mild oxidation conditions was used. Transitions to the deglycosylated peptide were monitored in prostate cancer tissues ($n = 9$) and non-cancer tissues ($n = 9$). While no differentially expressed levels of the deglycosylated peptide were observed using the global enrichment method, higher levels of the deglycosylated PSA peptide were observed when the sialic acid-specific enrichment method was used suggesting, albeit indirectly, increased sialylation of PSA in prostate cancer [24]. More recently, SPEG in combination with MRM was employed for the identification of differentially expressed glycopeptides in serum of esophageal adenocarcinoma (EAC) patients [25]. Here, five glycopeptides, originating from four glycoproteins were identified to be differentially expressed.

The use of lectins for glycan-specific enrichment of glycoproteins in combination with MRM has also been performed. Aberrantly glycosylated TIMP-1 has previously been associated with colorectal cancer. To quantify the differentially glycosylated TIMP-1, the protein was enriched using phytohemagglutinin-L (L-PHA)—a lectin that specifically recognizes the beta-1,6-GlcNAc moiety of N-linked glycans, followed by Stable Isotope Standard Capture with Anti-Peptide Antibodies (SISCAPA) and MRM [26]. Using this method, the aberrantly glycosylated TIMP-1 was quantified from less than 2 μL of serum. The method was subsequently extended to cover a larger fraction of the serum

glycoproteins using the four lectins AAL, L-PHA, ConA, and DSA. MRM transitions were developed for peptides originating from 67 proteins [27]. An average coefficient of variation (CV) of 7.9 % was reported for the higher abundance proteins monitored in the study, but no clinical application was reported.

The validation of differentially fucosylated glycoproteins that were previously identified was assessed in a similar fashion [28]. Using AAL lectin, followed by tryptic digestion and MRM analysis of the respective peptides, the four proteins APCS, C9, SERPINA4, and PON1 were shown to be differentially fucosylated in sera of non-small cell lung cancer patients [28] as illustrated in Fig. 1 for PON1.

An alternative strategy for the use of MRM in glycoprotein analysis is based on the observation that differential glycosylation may result in altered levels of missed cleavages [29, 30]. In studies by Lee et al. [30, 31], this phenomenon was used by monitoring the partially missed cleaved peptides. Lower levels of these peptides were associated with hepatocellular carcinoma (HCC) [30].

Quantification of Glycopeptides

Another strategy for quantitating glycosylation using MRM involves the direct analysis of glycopeptides. These methods are inherently difficult due to the lower ionization efficiencies induced by the glycan moiety on the peptide. Additionally, the heterogeneity of the glycosylation divides the intensities of the glycopeptides across several glycoforms.

The first step in this process is to determine the occupancy of a specific glycosite. Here, MRM can also be employed [32] by performing PNGaseF in the presence of ^{18}O yielding a labeled aspartic acid. MRM transitions developed to both the asparagine-containing peptide and the labeled aspartic acid are then used to determine the degree of occupancy. Using this method, it was shown that increased activity of congenital disease of glycosylation (CDG) type 1 results in a lower N-glycan occupancy [32].

So far, only a limited number of studies on the analysis of N-glycopeptides by MRM have been published [33–37]. In the majority of these studies, characteristic oxonium ions representing hexose (m/z 163), HexNAc (m/z 204), NeuAc (m/z 292), HexHexNAc (m/z 366), HexHexNAc-Fuc (m/z 512), and HexHexNAcNeuAc (m/z 657), with or without loss of water were used as the reporter ions. Only one study, in which the glycan moiety was stabilized using PA-labeling, reported the use of the peptide + HexNAc as the most intense reporter ion [36].

Biofluids, particularly serum or plasma, are typically the most readily available patient samples. All reported studies on the analysis of glycopeptides with MRM have, therefore, focused on these substances. We recently developed a method for the direct analysis of immunoglobulin G (IgG) and its glycoforms from serum [34]. The source parameters were specifically optimized for glycans and glycopeptides, a necessary procedure as these compounds are relatively more labile than the peptides and small molecules—the standard compounds used by manufacturers for optimizing commercial QQQ instruments. Transitions were developed for 26 glycopeptides from IgG to the oxonium ions m/z 366 (HexHexNAc) or m/z 204 (HexNAc). To achieve normalization of the glycosylation profile to the overall protein content, transitions were also developed for non-glycosylated peptides. The collision energies were also optimized. The resulting method yielded a limit of detection of 60 amol and a wide dynamic range extending 3 orders magnitude for IgG protein quantitation [34]. A typical MRM chromatogram of IgG is shown in Fig. 2. The glycopeptides of IgG subclass 3 and IgG subclass 4 could not be distinguished using this method directly; however, subclass-specific analysis can be obtained during the sample preparation [38]. This method is currently being expanded to the other immunoglobulins IgA and IgM (manuscript in preparation). Altered glycosylation of immunoglobulins has been associated with multiple diseases [39], and the differential immunoglobulin glycosylation of ovarian cancer is currently being assessed using MRM (manuscript in preparation).

A similar approach was used for the differential analysis of haptoglobin in plasma of HCC patients [35]. Fucosylation of haptoglobin is increased in HCC [40] and upon isolation of HCC and treatment with sialidase and galactosidase, 24 glycopeptides of the T3-glycopeptide containing N-glycosylation site N^{241} were monitored. Transitions were developed to oxonium ions at m/z 204, m/z 366, and m/z 512 and the collision energies were optimized. Using this method, differential fucosylation of this specific haptoglobin glycopeptide in HCC patients compared to controls and cirrhosis patients was determined [35].

An alternative, not protein specific, approach has been employed by Kurogochi et al. [36], who aimed to quantify a broad range of sialylated glycopeptides from mouse serum. To enrich for sialylated glycopeptides, a reverse glycoblotting technique was used, which allows for the retrieval of glycopeptides as pyridylaminated conjugates. The enriched glycopeptides were then characterized using LC-MS/MS, followed by the development of MRM transitions. Interestingly, the peptide + HexNAc fragment was identified as the most important reporter ion. Using this approach, 67 glycopeptides from mouse serum could be monitored and using a diabetes disease model, differently expressed glycopeptides were identified [36].

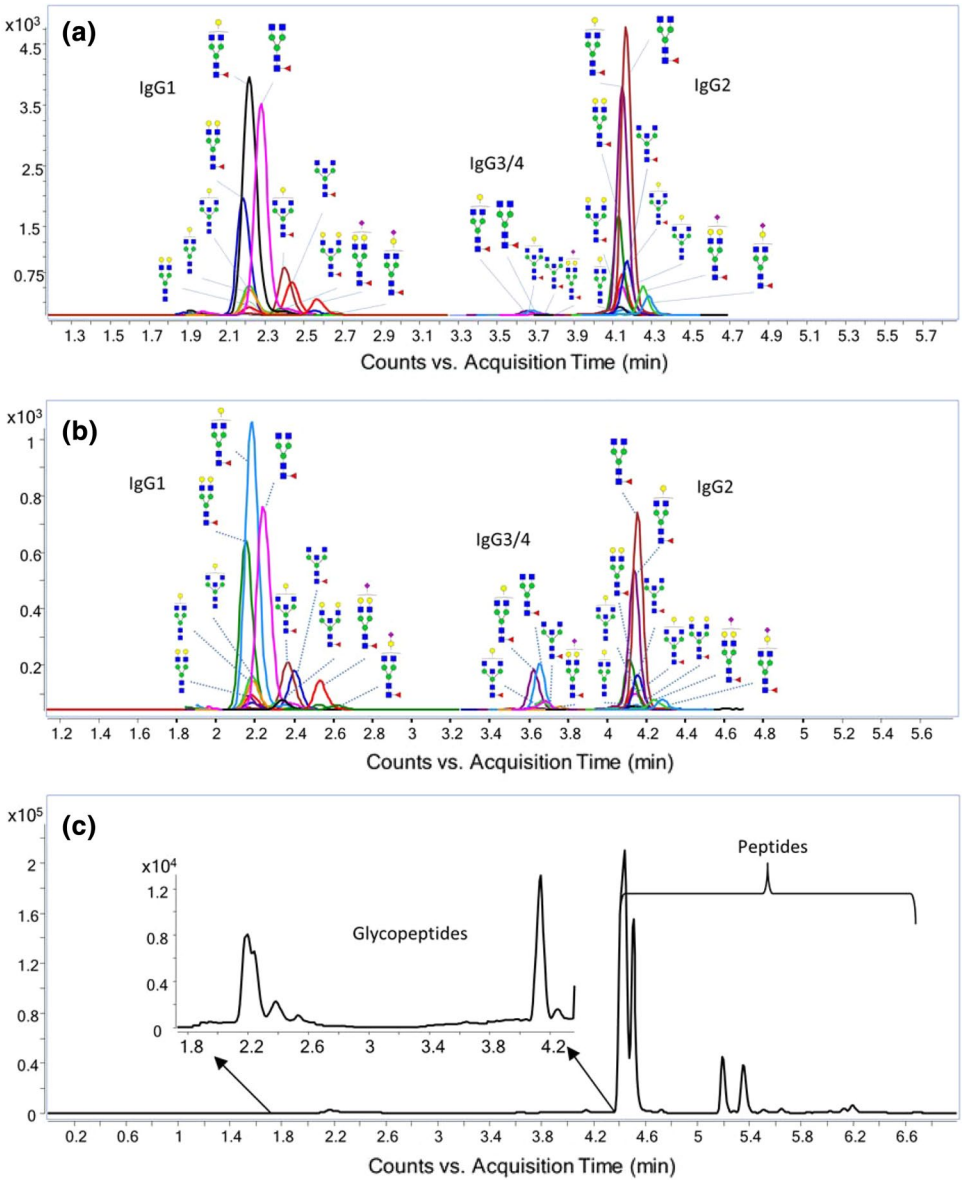

Fig. 2 MRM chromatograms of immunoglobulin G and its glycopeptides. Depicted are the chromatograms of **a** glycopeptides in a tryptic digest of IgG standard **b** glycopeptides in a tryptic digest of serum and **c** peptides and glycopeptides from a tryptic digest of serum. One MRM transition was monitored for each glycopeptide with either the HexNAc or HexHexNAc oxonium ion as the reporter ion; two MRM transitions were monitored for each peptide. Reprinted with permission from [34] Copyright 2013 American Chemical Society

A similar approach was recently used by Song et al. who first applied lectin enrichment using an SNA and AAL mixture on high abundant protein-depleted human serum samples, and then used MRM for glycopeptide quantifications [25]. Transitions for 57 glycopeptides were monitored to the characteristic oxonium ions at m/z 204, 274, and 366. The method was then applied to serum samples from EAC patients, patients with high-grade dysplasia (HGD) in the esophagus, and disease-free controls (DF). Four glycopeptides were identified to be differentially expressed in EAC compared to DF, while levels of 11 glycopeptides were significantly altered between HGD and DF.

The analysis of O-GlcNAc modifications is inherently difficult. Recently, the successful use of MRM for the identification of an O-GlcNAcylated peptide was reported [41]. A commercially available O-GlcNAcylated peptide was used to optimize instrument parameters and show the feasibility of LC-MRM-MS for the quantitation of the O-GlcNAc modification. Characteristic peptide fragments were shown to be most suitable for quantification. GSK-3beta is known to be O-GlcNAcylated, but the site of attachment is not clear. Using LC-MRM, the O-GlcNAcylated peptide could be identified unambiguously [41].

Quantification of Oligosaccharides

One of the earliest applications of MRM for oligosaccharide analysis was in the analysis of a glycogen-derived glucose tetrasaccharide that is known to be elevated in urine

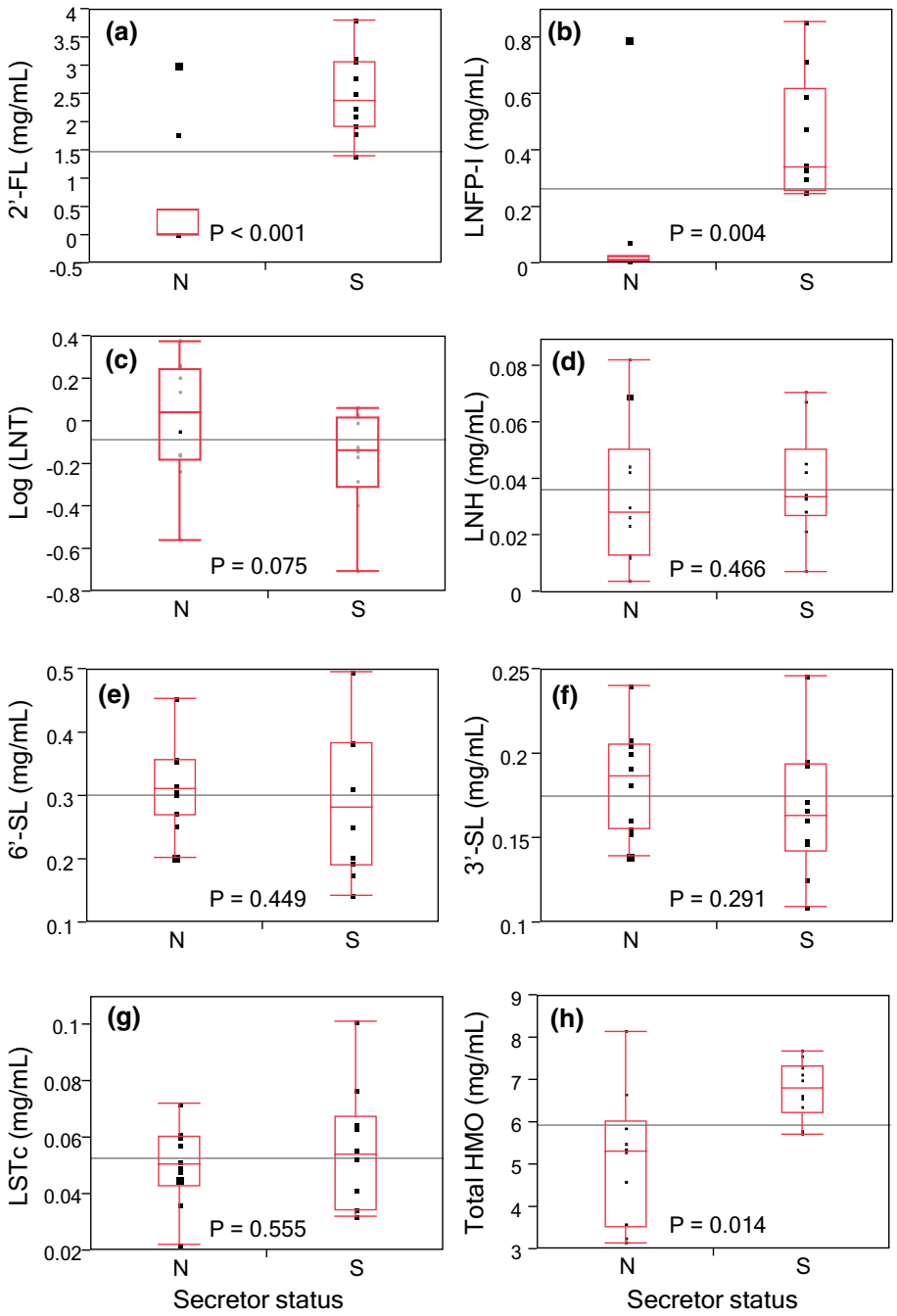

Fig. 3 The profile of human milk oligosaccharides in a mother's milk is dependent on her secretor status. Shown are box-whisker plots for secretor mothers (S) and non-sectretor mothers (N). Plots are depicted for **a** 2′-FL, **b** LNFP-I, **c** LNT, **d** LNH, **e** 6′-SL, **f** 3′-SL, **g** LSTc, and **h** Total HMO content, and the reported *P* values are from one-tail Student's *t* test unless specified. Values were obtained using integrals of MRM signals of native reduced HMO in the positive ionization mode. Reprinted with permission from [44]. Copyright 2014 American Chemical Society

of those with Pompe disease [42]. Butyl-4-aminobenzoate (BAB) derivatized oligosaccharides were analyzed, and a C_{13}-labeled tetraglucose standard was added during sample preparation to increase accuracy of quantification. Transitions were monitored toward the sodium adduct of Glc_3 and the results obtained with the LC-MRM method were in good agreement with the conventional HPLC–UV method, indicating that MRM can be used for the analysis of oligosaccharides for the determination of Pompe disease.

Four additional studies have recently been reported on the use of MRM for the quantitation of human oligosaccharides [43–45], each presenting alternative methods. We recently reported on the use of MRM for the analysis and quantification of human milk oligosaccharides (HMO) in the native, reduced state. Using a porous graphitized carbon stationary phase, structural isomers can be separated and quantified individually, resulting in the simultaneous analysis of 49 HMO structures. Seven standard HMO (2′-FL, LNT, LNFP-I, 3′-SL, 6′-SL, and LSTc) were used for absolute quantitation and the levels of detection for each of these compounds was found to be in the low femtomole to attomole range. Using this method,

significant differences were observed in the HMO composition of secretors (mothers that can excrete HMO with α1–2-linked fucose) and non-secretors (mothers that do not produce HMO with α1–2-linked fucose) [44] as is illustrated in Fig. 3.

Two alternative strategies that are based on labeling techniques have been applied to oligosaccharides. 1-phenyl-3-methyl-5-pyrazolone (PMP) labeling has been reported to be instrumental in the quantification of urinary oligosaccharides [43], Under CID, a characteristic PMP-fragment at m/z 175 was consistently observed in the product ion scans, which was used as the reporter ion. Interestingly, the PMP-specific stationary phase pentafluorophenylpropyl was used, and good separation was observed. Using this method, signals characteristic of CDGs were observed in the urine of CDG patients [43]. An alternative strategy for relative quantification using labeling with alanine and D_6-labeled alanine was reported for N-glycan analysis [45]. When the D_6-alanine labeled standards were used, better CVs of less than 5 % were obtained compared to quantitation without heavy labeling. 42 N-glycans were observed to be differentially expressed in serum from rabbit on either a high cholesterol diet ($n = 5$) or a control diet ($n = 5$) [45].

Permethylation has been widely applied in mass spectrometry analysis of released glycans to stabilize sialic acid linkages and improve fragmentation efficiency. MRM was recently employed for the analysis of permethylated N-glycans released from serum proteins from two cohorts of HCC cases and liver cirrhosis controls [46]. Next to global profiling using LC-MS, transitions were developed for 117 N-glycans on QQQ-MS. using MRM, 15 glycans were shown to be differentially expressed in one of the two cohorts, of which 11 overlapped with the global profiling method. None of the glycans was identified to be differentially expressed in both cohorts.

To allow absolute quantitation of oligosaccharides, standards are needed. There are some standards that are commercially available (mostly N-glycans and milk oligosaccharides), but they are typically very expensive and cover only a small range of the glycan heterogeneity. To overcome this, we determined the average response factor of seven HMO standards and applied that to all milk oligosaccharides in a mother's milk [44]. The overall total HMO amount thus calculated was in good concordance with the actual amount, indicating that such a generalization may provide results that are quite accurate.

Conclusions and Future Prospects

Multiple reaction monitoring is a technique that is widely acknowledged for its accurate quantitation, which is an area in glycomics that could still benefit from further methodological improvements. MRM can be used only after the identification of a compound in a given biological process which is typically performed using other mass spectrometry-based detection techniques such as time-of-flight or ion trap mass spectrometry. Quantitation with validation of the compound is then performed using MRM. A benefit of this strategy is that it allows for the identification of the typical charge state and characteristic fragment ions during the identification process, which can then be used in the development of the MRM transitions, thereby enabling faster method development of MRM analysis.

Application of MRM in the field of glycomics can be subdivided in three areas: quantification of glycoproteins, glycopeptides, and oligosaccharides, with each of them having their intrinsic difficulties. When quantifying glycoproteins, the selection of glycopeptides for monitoring is of importance [47]. Most importantly, one should take the presence of amino acid modifications such as oxidation of methionine and deamidation of glutamine and asparagine into account. If possible, peptides containing such modifications should be avoided, as their quantitation is typically less accurate.

The choice of protease also largely influences the MRM analysis of both glycoproteins and glycopeptides [48, 49]. Typically tryptic digestion is used for the generation of peptides or glycopeptides, but, due to the uneven distribution of lysine and arginine residues this may result in long peptides that are not readily detectable by MS. Furthermore, when analyzing glycopeptides, such long peptides can result in doubly glycosylated glycopeptides thereby complicating the resolution of the glycosylation profile. This was observed for one of the tryptic glycopeptides from haptoglobin, and one of the reasons why it was decided to monitor the glycosylation profile on one of the other glycopeptides [35]. However, such a strategy cannot be typically employed, as the glycosylation pattern is different by site of glycosylation. Therefore, the use of alternative proteases should be considered, as was illustrated by Maury et al. who used Glu-C to generate O-GlcNAcylated glycopeptides [41].

Labeled standards are often used in mass spectrometry to increase the accuracy of both absolute and relative quantitation. Such labels are now often multiplexed and isobaric, but provide characteristic fragment ions that differ in mass by 1 da (e.g., [50]). However, due to the large inclusion windows of the quadrupoles (typically at least 0.5 da), these strategies are not suitable in MRM. Alternative strategies may include the use of non-isobaric labeling [51], and this has been applied in MRM. A D_6-labeled alanine was used for the labeling of glycans, resulting in a Δm of 6 da, which was shown to be largely sufficient in MRM [45]. Similar approaches for peptide and glycopeptide analysis

are available and would be suitable for use in MRM-based quantitation.

Compared to peptides and metabolites, the MRM of glycans and glycoconjugates is still in its infancy. Thus far, there have been limited clinical applications of MRM in the glycomics field; however, the initial studies using MRM have shown its potential for clinical applications and especially in the validation of earlier findings. While the lack of standards, both glycans and glycoconjugates, is a major issue, which hinders the field, the applications of MRM within the field of glycomics are expected to increase as its utility becomes better known. It is, therefore, anticipated that MRM will play a more profound role in clinical glycomics applications in the near future.

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References

1. Shental-Bechor D, Levy Y (2009) Curr Opin Struct Biol 19:524–533. doi:10.1016/j.sbi.2009.07.002
2. Fukuda MN, Sasaki H, Lopez L, Fukuda M (1989) Blood 73:84–89
3. Gu J, Isaji T, Xu Q, Kariya Y, Gu W, Fukuda T, Du Y (2012) Glycoconj J 29:599–607. doi:10.1007/s10719-012-9386-1
4. Ruhaak LR, Miyamoto S, Lebrilla CB (2013) Mol Cell Proteomics 12:846–855. doi:10.1074/mcp.R112.026799
5. Kim K, Ruhaak LR, Nguyen UT, Taylor SL, Dimapasoc L, Williams C, Stroble C, Ozcan S, Miyamoto S, Lebrilla CB, Leiserowitz GS (2014) Cancer Epidemiol Biomark Prev 23:611–621. doi:10.1158/1055-9965.EPI-13-1073
6. Ozcan S, Barkauskas DA, Renee RL, Torres J, Cooke CL, An HJ, Hua S, Williams CC, Dimapasoc LM, Han KJ, Camorlinga-Ponce M, Rocke D, Lebrilla CB, Solnick JV (2014) Cancer Prev Res (Phila) 7:226–235. doi:10.1158/1940-6207.CAPR-13-0235
7. Adamczyk B, Tharmalingam T, Rudd PM (2012) Biochim Biophys Acta 1820:1347–1353. doi:10.1016/j.bbagen.2011.12.001
8. Alley WR Jr, Vasseur JA, Goetz JA, Svoboda M, Mann BF, Matei DE, Menning N, Hussein A, Mechref Y, Novotny MV (2012) J Proteome Res 11:2282–2300. doi:10.1021/pr201070k
9. Selman MHJ, Niks EH, Titulaer MJ, Verschuuren JJGM, Wuhrer M, Deelder AM (2011) J Proteome Res 10:143–152. doi:10.1021/Pr1004373
10. Parekh RB, Dwek RA, Sutton BJ, Fernandes DL, Leung A, Stanworth D, Rademacher TW, Mizuochi T, Taniguchi T, Matsuta K et al (1985) Nature 316:452–457
11. Itoh N, Sakaue S, Nakagawa H, Kurogochi M, Ohira H, Deguchi K, Nishimura S, Nishimura M (2007) Am J Physiol Endocrinol Metab 293:E1069–E1077. doi:10.1152/ajpendo.00182.2007
12. Ruhaak LR, Uh HW, Beekman M, Hokke CH, Westendorp RG, Houwing-Duistermaat J, Wuhrer M, Deelder AM, Slagboom PE (2011) J Proteome Res 10:1667–1674. doi:10.1021/pr1009959
13. Vanhooren V, Desmyter L, Liu XE, Cardelli M, Franceschi C, Federico A, Libert C, Laroy W, Dewaele S, Contreras R, Chen C (2007) Rejuvenation Res 10:521a–531a. doi:10.1089/rej.2007.0556
14. Ruhaak LR, Uh HW, Deelder AM, Dolhain RE, Wuhrer M (2014) J Proteome Res 13:1657–1668. doi:10.1021/pr401128j
15. Bondt A, Selman MH, Deelder AM, Hazes JM, Willemsen SP, Wuhrer M, Dolhain RJ (2013) J Proteome Res 12:4522–4531. doi:10.1021/pr400589m
16. Kuzyk MA, Smith D, Yang J, Cross TJ, Jackson AM, Hardie DB, Anderson NL, Borchers CH (2009) Mol Cell Proteomics 8:1860–1877. doi:10.1074/mcp.M800540-MCP200
17. Wei R, Li G, Seymour AB (2010) Anal Chem 82:5527–5533. doi:10.1021/ac100331b
18. An HJ, Lebrilla CB (2010) Mass Spectrom Rev. doi:10.1002/mas.20283
19. Wuhrer M, Catalina MI, Deelder AM, Hokke CH (2007) J Chromatogr B Analyt Technol Biomed Life Sci 849:115–128. doi:10.1016/j.jchromb.2006.09.041
20. Kolli V, Dodds ED (2014) Analyst 139:2144–2153. doi:10.1039/c3an02342g
21. Tian Y, Zhou Y, Elliott S, Aebersold R, Zhang H (2007) Nat Protoc 2:334–339. doi:10.1038/nprot.2007.42
22. Lee A, Nakano M, Hincapie M, Kolarich D, Baker MS, Hancock WS, Packer NH (2010) OMICS 14:487–499. doi:10.1089/omi.2010.0075
23. Kim YJ, Zaidi-Ainouch Z, Gallien S, Domon B (2012) Nat Protoc 7:859–871. doi:10.1038/nprot.2012.023
24. Li Y, Tian Y, Rezai T, Prakash A, Lopez MF, Chan DW, Zhang H (2011) Anal Chem 83:240–245. doi:10.1021/ac102319g
25. Song E, Zhu R, Hammoud ZT, Mechref Y (2014) J Proteome Res. doi:10.1021/pr500570m
26. Ahn YH, Lee JY, Lee JY, Kim YS, Ko JH, Yoo JS (2009) J Proteome Res 8:4216–4224. doi:10.1021/pr900269s
27. Ahn YH, Ji ES, Shin PM, Kim KH, Kim YS, Ko JH, Yoo JS (2012) Analyst 137:691–703. doi:10.1039/c1an15775b
28. Ahn JM, Sung HJ, Yoon YH, Kim BG, Yang WS, Lee C, Park HM, Kim BJ, Kim BG, Lee SY, An HJ, Cho JY (2014) Mol Cell Proteomics 13:30–48. doi:10.1074/mcp.M113.028621
29. An HJ, Peavy TR, Hedrick JL, Lebrilla CB (2003) Anal Chem 75:5628–5637
30. Lee JY, Kim JY, Park GW, Cheon MH, Kwon KH, Ahn YH, Moon MH, Lee HJ, Paik YK, Yoo JS (2011) Mol Cell Proteomics 10(M111):009290. doi:10.1074/mcp.M111.009290
31. Lee JY, Kim JY, Cheon MH, Park GW, Ahn YH, Moon MH, Yoo JS (2014) J Proteomics 98C:206–217. doi:10.1016/j.jprot.2014.01.003
32. Hulsmeier AJ, Paesold-Burda P, Hennet T (2007) Mol Cell Proteomics 6:2132–2138. doi:10.1074/mcp.M700361-MCP200
33. Song E, Pyreddy S, Mechref Y (2012) Rapid Commun Mass Spectrom RCM 26:1941–1954. doi:10.1002/rcm.6290
34. Hong Q, Lebrilla CB, Miyamoto S, Ruhaak LR (2013) Anal Chem 85:8585–8593. doi:10.1021/ac4009995
35. Sanda M, Pompach P, Brnakova Z, Wu J, Makambi K, Goldman R (2013) Mol Cell Proteomics 12:1294–1305. doi:10.1074/mcp.M112.023325
36. Kurogochi M, Matsushista T, Amano M, Furukawa J, Shinohara Y, Aoshima M, Nishimura S (2010) Mol Cell Proteomics 9:2354–2368. doi:10.1074/mcp.M110.000430
37. Zhao Y, Jia W, Wang J, Ying W, Zhang Y, Qian X (2011) Anal Chem 83:8802–8809. doi:10.1021/ac201676a
38. Wuhrer M, Stam JC, van de Geijn FE, Koeleman CA, Verrips CT, Dolhain RJ, Hokke CH, Deelder AM (2007) Proteomics 7:4070–4081. doi:10.1002/pmic.200700289
39. Huhn C, Selman MH, Ruhaak LR, Deelder AM, Wuhrer M (2009) Proteomics 9:882–913. doi:10.1002/pmic.200800715
40. Ang IL, Poon TC, Lai PB, Chan AT, Ngai SM, Hui AY, Johnson PJ, Sung JJ (2006) J Proteome Res 5:2691–2700. doi:10.1021/pr060109r

41. Maury JJ, Ng D, Bi X, Bardor M, Choo AB (2014) Anal Chem 86:395–402. doi:10.1021/ac401821d
42. Young SP, Stevens RD, An Y, Chen YT, Millington DS (2003) Anal Biochem 316:175–180
43. Sowell J, Wood T (2011) Anal Chim Acta 686:102–106. doi:10.1016/j.aca.2010.11.047
44. Hong Q, Ruhaak LR, Totten SM, Smilowitz JT, German JB, Lebrilla CB (2014) Anal Chem 86:2640–2647. doi:10.1021/ac404006z
45. Zhang H, Wang Z, Stupak J, Ghribi O, Geiger JD, Liu QY, Li J (2012) Proteomics 12:2510–2522. doi:10.1002/pmic.201100567
46. Tsai TH, Wang M, Di Poto C, Hu Y, Zhou S, Zhao Y, Varghese RS, Luo Y, Tadesse MG, Ziada DH, Desai CS, Shetty K, Mechref Y, Ressom HW (2014) J Proteome Res. doi:10.1021/pr500460k
47. Lange V, Picotti P, Domon B, Aebersold R (2008) Mol Syst Biol 4:222. doi:10.1038/msb.2008.61
48. Choudhary G, Wu SL, Shieh P, Hancock WS (2003) J Proteome Res 2:59–67
49. Wisniewski JR, Mann M (2012) Anal Chem 84:2631–2637. doi:10.1021/ac300006b
50. Unwin RD (2010) Methods Mol Biol 658:205–215. doi:10.1007/978-1-60761-780-8_12
51. Lengqvist J, Sandberg A (2013) Methods Mol Biol 1023:21–51. doi:10.1007/978-1-4614-7209-4_3