Innovative direct introduction-ion mobility–mass spectrometry (DI-IM-MS) approach for fast and robust isomer-specific quantification in a complex matrix: Application to 2'-fucosyllactose (2'-FL) in breast milk

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Innovative direct introduction-ion mobility–mass spectrometry (DI-IM-MS) approach for fast and robust isomer-specific quantification in a complex matrix: Application to 2′-fucosyllactose (2'-FL) in breast milk

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Abstract

Identification and specific quantification of isomers in a complex biological matrix by mass spectrometry alone is not an easy task due to their identical chemical formula and therefore their same mass-to-charge ratio (*m/z*). Here, the potential of direct introduction combined with ion mobility–mass spectrometry (DI-IM-MS) for rapid quantification of isomers as human milk oligosaccharides (HMOs) was investigated. Differences in HMO profiles between various analyzed breast milk samples were highlighted using the single ion mobility monitoring (SIM^2) acquisition for high ion mobility resolution detection. Furthermore, the Se+ (secretor) or Se− (non-secretor) phenotype could be assigned to breast milk samples studied based on their HMO contents, especially on the response of 2′-fucosyllactose (2'-FL) and lacto-N-fucopentaose I (LNFP I). The possibility of quantifying a specific isomer in breast milk by DI-IM-MS was also investigated. The standard addition method allowed the determination of the 2'-FL despite the presence of other oligosaccharides, including 3-fucosyllactose (3-FL) isomer in breast milk. This proof-of-concept study demonstrated the high potential of such an approach for the rapid and convenient quantification of isomers in complex mixtures.

KEYWORDS
direct introduction, human milk oligosaccharides (HMOs), ion mobility–mass spectrometry (IM-MS), isomers, quantification

1 | INTRODUCTION

The quantification of isomers in biological matrices represents a real analytical challenge for various scientific fields, either in the environment or life sciences. Besides matrix effects, the low concentration of the analyte and the absence of a true blank matrix to prepare calibration solutions, one of the major problems in quantitative analysis is the separation of the isomers to be quantified. Despite advances in analytical techniques, high-performance instrumentation like high-resolution mass spectrometry (HRMS) instruments cannot alone resolve structural isomers due to their identical chemical formula and therefore their identical masses. Various hyphenated techniques have been

developed to overcome this issue, notably by coupling a separation system such as liquid chromatography (LC), gas chromatography (GC), or capillary electrophoresis (CE) with a mass spectrometer.[1] However, a major limitation of such approaches is their complexity related to the analytical conditions to be set up, which drastically increases the analysis cost and slows down the analytical process. Additional tandem mass spectrometric techniques using collision-induced dissociation (CID) are often performed to monitor diagnostic fragment ions for specific quantification purposes.[2] The efficacy of the latter approaches highly depends on the fragmentation pathways of the parent ions which must differ sufficiently to observe significant differences in their tandem mass spectra. An ideal analytical method should be fast, sensitive, selective, and robust to provide reliable and quantitative data.

Unlike the conventional chromatographic techniques, ion mobility (IM) separation based on gas-phase IM rather than polarity is not limited by solvent or stationary-phase constraints.[3] Its coupling with mass spectrometry (IM-MS) offers an additional separation dimension without lengthening the MS acquisition time. In addition, its potential in characterizing isomers that are not easily separated by LC or GC techniques has been demonstrated in various analytical fields such as pharmaceuticals[4] and omics areas.[5] To our knowledge, there are few bioanalytical methods based on IM-MS technology developed to date for the rapid quantification of isomers in complex matrices by direct introduction. Most studies have reported the use of direct infusion IM-MS for the relative quantification of many compound classes, for example, measurements of isomeric macromolecules as polymers within a synthetic mixture[6] or determination of enantiomeric ratios in chiral IM analysis.[7,8] Only, differential mobility spectrometry (DMS) hyphenated to tandem mass spectrometry (MS/MS) has been used for the direct quantification of amino acids and related compounds.[9]

In this study, the feasibility of combining direct introduction by flow injection analysis (FIA) with IM-MS to quantify isomers in a complex mixture was investigated. As breast milk is composed of many structurally diverse oligosaccharides, including many structural isomers,[10] this complex matrix appeared ideally suited to assess the relevance of isomer-specific quantification by FIA-IM-MS. First, the relative abundances of selected human milk oligosaccharides (HMOs) were estimated based on their IM-MS responses. Then, two quantitative approaches, external standard calibration and standard addition methods were explored for measuring the content of a specific isomer of HMOs, here 2′-fucosyllactose (2'-FL). Despite the presence of various oligosaccharide isomers in breast milk, the determination of 2'-FL could be achieved thanks to the single ion mobility monitoring (SIM^2) mode, which enabled its specific detection at high IM resolving power.[11,12]

2 | MATERIALS AND METHODS

2.1 | Chemicals and breast milk samples

ESI-L Low Concentration Tuning Mix (G1969-85000), a mass and mobility calibration solution, was obtained from Agilent Technologies (Santa Clara, CA, USA). High-performance liquid chromatography (HPLC)-grade methanol was acquired from VWR Chemicals (Fontenay-sous-Bois, France). Ultrapure water with a resistivity of 18.2 MΩ·cm was produced by a Select HP water purification system (France Eau, Lormont, France). Most of the HMO standards were purchased from Carbosynth (Bratislava, Slovakia) such as 2'-FL, 3-fucosyllactose (3-FL), 3′-sialyllactose (3'-SL), 6′-sialyllactose (6'-SL), lacto-N-fucopentaose I (LNFP I) and II (LNFP II), sialyllacto-N-tetraose b (LSTb) and sialyllacto-N-neotetraose c (LSTc), lactodifucotetraose (LDFT), and lacto-N-difucohexaose I (LNDFH I), while lacto-N-tetraose (LNT), lacto-N-neotetraose (LNnT), sialyllacto-N-tetraose a (LSTa), LNFP V and VI, and LNDFH II came from Elicityl (Crolles, France). Their structures are shown in Table S1. The five breast milk samples studied here were randomly selected from samples collected for the previous study from healthy donor mothers and stored at −80°C before use.[13]

2.2 | Preparation of calibration solutions and breast milk samples

The stock solution of 2'-FL calibration standard at a concentration of 100 ng.μL^{-1} was obtained by dissolving accurately weighed standard in water–methanol (1:1, v/v). A working solution of 10 ng.μL^{-1} obtained by diluting 10 times the stock solution of 2'-FL was used to prepare calibration solutions in the concentration range of 0.05–1 ng.μL^{-1} in water–methanol (1:1, v/v) and in the concentration range of 0.01–1 ng.μL^{-1} for spiked matrix solutions.

Breast milk samples were prepared as follows[14]: a 1:10 dilution was made by mixing 50 μL of a breast milk sample with 450 μL of cold water–methanol (4:6, v/v). The resulting mixture was stored at −80°C for at least 2 h and then centrifuged at 3000 ×g at 4°C for 30 min to separate the precipitated proteins. The upper liquid phase was collected and further diluted in water–methanol (1:1, v/v) before being analyzed by IM-MS. Note that another sample preparation method aimed at separating lipids (top layer) by adding water and centrifuging the resulting sample was also tested.[15] The obtained extract provided similar results in terms of HMO profiles and their contents.

Both unspiked and diluted breast milk samples spiked at different concentrations of the 2'-FL standard were analyzed by direct introduction-trapped ion mobility spectrometry (TIMS) time-of-flight (TOF) mass spectrometry.

2.3 | Ion mobility spectrometry–mass spectrometry analysis

All IM-MS experiments were performed on a trapped IM spectrometer—quadrupole TOF (Bruker Daltonics, Bremen, Germany)[16] equipped with an electrospray ionization (ESI) source. FIA was performed to automate sample injections with a flow rate of 50 μL.min^{-1} of water–methanol (1:1, v/v) using only the autosampler of an HPLC system (Elute, Bruker Daltonics). The instrumental parameters were optimized for the detection of HMOs using the oTof

control software (Bruker Daltonics). The end plate offset was set at 500 V and the electrospray voltage at −4500 and +3500 V in positive and negative ionization, respectively. The capillary temperature was maintained at 250°C. Nitrogen was used as the spray and drift gas. The dry gas and the nebulizer gas were fixed at 3.0 L.min^{-1} and 0.3 bar, respectively. In the TIMS analyzer, the Funnel 1 RF, the Funnel 2 RF, and the deflection delta were set at 260 Vpp, 250 Vpp, and ±80 V, respectively. The ion charge control (ICC) was kept at 1.5 Mio to avoid TIMS saturation.

External calibrations (in quadratic mode) of the mass-to-charge ratio (*m/z*) and reduced mobility values (in linear mode) were carried out before experiments using the ESI-L Low Concentration Tuning Mix. Mass spectra were recorded in the 100–1650 *m/z* range with a transfer time of 70 µs and a pre-pulse storage of 5 µs. Two IM detection modes were used: (i) a full IM scan using a large IM range with inverse reduced mobility ratios ($1/K_0$) ranging from 0.55 to 1.90 V·s·cm^{-2} and a scan rate of about 9 Hz, and (ii) SIM^2 mode using a narrow mobility range, typically a $1/K_0$ window of about 0.10 V·s·cm^{-2} with a scan rate between 1 and 3 Hz.

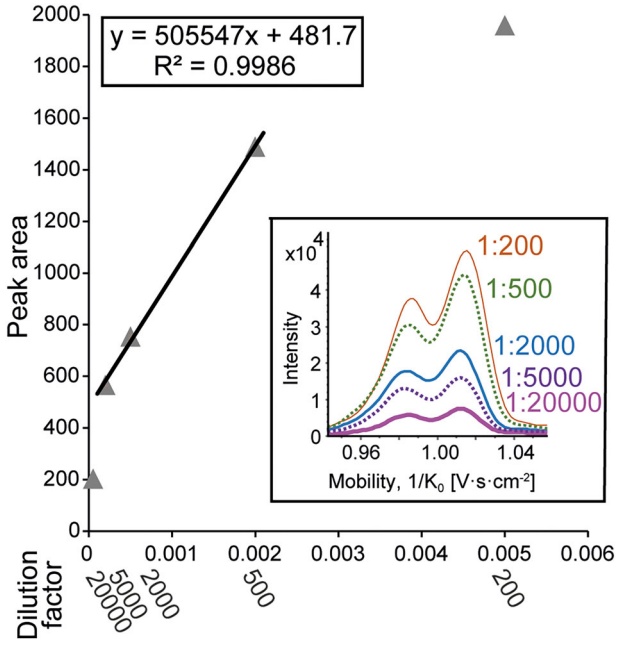

FIGURE 1 Ion mobility–mass spectrometry (IM-MS) analysis of the breast milk sample BM1 diluted at five dilution factors (i.e., 200, 500, 2000, 5000, and 20 000) using full ion mobility detection: plot of the sum of the peak areas (i.e., areas of the two peaks not separated) as a function of the dilution factor and, in the inset, the variation of the ion mobility signal corresponding to the *m/z* 487.17 $[M-H]^-$ of 2'-FL and/or 3-FL isomers as a function of the dilution factor. Note that the values on the *x*-axis correspond to the dilution values, for example, a value of 0.002 (=1:500) corresponds to a dilution of 1:500.

3 | RESULTS AND DISCUSSION

In this study, the potential of TIMS to produce quantitative data was evaluated. First, it is necessary to determine an adequate dilution factor in order to minimize matrix effects that could impair the detection of the analyte of interest. This dilution factor must be within the linearity range of the analyte response. Indeed, the matrix effects being of the same order in this linearity range, a quantification by standard addition can be carried out reliably while a systemic bias can be created outside this range, by an over- or an under-estimation of the measured values, which is not expected from an assay. Here, breast milk samples were prepared at different dilutions (i.e., 1:200, 1:500, 1:2000, 1:5000, and 1:20000) and were directly analyzed by FIA-IM-MS using first full IM detection (i.e., a wide IM range, typically from 0.55 to 1.90 V·s·cm^{-2}). Most of the abundant HMOs (e.g., 2'-FL/3-FL and LNT) were detected, and the intensity of their IM signals varied as a function of the dilution factor applied (Figures 1 and S1, not all data shown). Indeed, a linear response was observed for the *m/z* 487 $[M-H]^-$ species of 2'-FL and/or 3-FL in the dilution range of 1:500 to 1:5000 with a determination coefficient R^2 of 0.9986 (Figure 1). Conversely, their signal was quite weak at 1:5000 and 1:20000 dilution, but remained detectable. The loss of the linearity at lower dilution (i.e., 1:200) could probably be due to matrix effects caused by the presence of other interfering breast milk components (e.g., lipids and organic residues) which may compete with the HMOs in the ESI process.[17,18] The dilution factor of 2000 falling in the middle of the linearity range seemed to be a good compromise for further analysis of breast milk, providing acceptable detection intensity and minimized matrix effects.

Then, a full IM detection allowing to cover as many compounds as possible in a single IM-MS acquisition was applied to analyze breast milk samples diluted at 1:2000. Different HMO profiles were observed, indicating a different HMO composition for every breast milk (Figures 2 and S2). For example, the unseparated isomers 2'-FL/3-FL, LNFPs, and LSTs were detected in all four breast milk samples analyzed, BM1 to BM4, but their signal intensity varied between samples, while LDFT and LNDFHs were only detected in the sample BM1 for a dilution of 1:2000. Most HMO isomers could not be resolved using this IM mode, with the exception of the well-separated LNT and LNnT, for which their contents could be compared in the four breast milk samples based on the intensity of their *m/z* 706 $[M-H]^-$ species (Figure S3).

A SIM^2 mode using narrow IM range detection (typically a $1/K_0$ window of about 0.10 V·s·cm^{-2}) can improve mobility separation of TIMS device but in a targeted manner. The efficacy of SIM^2 mode for the characterization and distinction of multiple HMO isomers has been demonstrated in our previous work.[11,12] Here, SIM^2 acquisition was applied to obtain more informative HMO profiles from complex breast milk samples. The possibility of distinguishing certain relevant HMO isomers thanks to the use of the SIM^2 mode made it possible to compare their response (e.g., the intensity of their IM peaks for a given species) to that of other HMOs within the same sample (intra-sample) but also the response of a given HMO between samples (inter-samples) (Figures 3 and S3). Such comparison showed different HMO patterns based on variations in the relative abundance of some

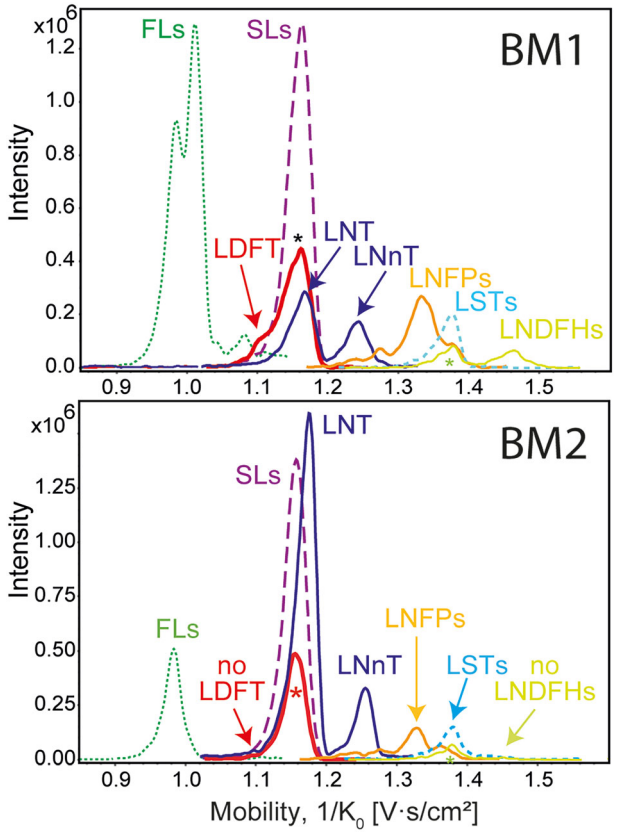

FIGURE 2 Ion mobility–mass spectrometry (IM-MS) analysis of two breast milk samples, BM1 and BM2, showing different human milk oligosaccharide (HMO) profiles through $[M-H]^-$ species signals of each HMO zoomed on their specific mobility ranges using full ion mobility detection. Fucosyllactose (FLs) for 2′-fucosyllactose (2'-FL)/3-fucosyllactose (3-FL), sialyllactose (SLs) for 3′-sialyllactose (3'-SL)/6′-sialyllactose (6'-SL), and so forth (see their structures in Table S1), the peaks marked with an asterisk (*) are isobars: (i) the isobar of the *m/z* 633.22 $[M-H]^-$ species of lactodifucotetraose (LDFT) could correspond to the ^{13}C isotope of the $[M-H]^-$ species of SLs and, (ii) the ^{13}C isotope of the $[M-H]^-$ species of sialyllacto-N-tetraose and sialyllacto-N-neotetraose (LSTs) could be detected as an isobar of the $[M-H]^-$ ions of lacto-N-difucohexaose (LNDFHs).

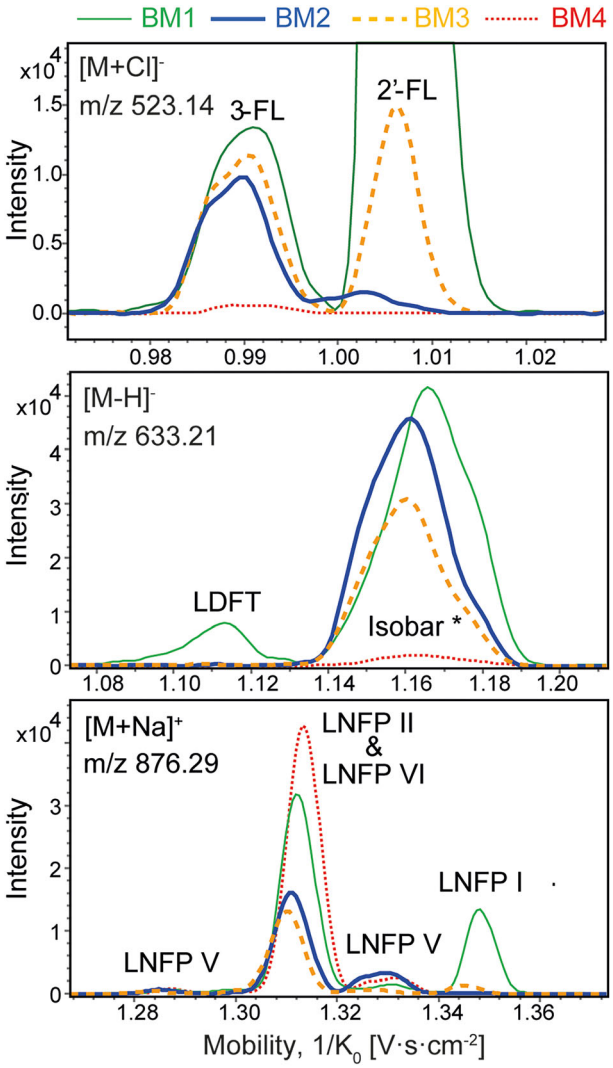

FIGURE 3 Ion mobility–mass spectrometry (IM-MS) analysis of four breast milk samples, BM1 to BM4, showing the separation or distinction of some human milk oligosaccharide (HMO) isomers using single ion mobility monitoring (SIM^2) acquisition.

selected HMO isomers, depending on the breast milk sample (Table 1). Indeed, the HMO composition varies from one mother to another, depending essentially on maternal genetics. Two genes, namely, the Secretor (Se) and Lewis (Le) genes, which code for the enzymes α1,2-fucosyltransferase 2 (FUT2) and α1,3/1,4-fucosyltransferase (FUT3), respectively, are responsible for HMO fucosylation.[19–21] There are four known existing genetic groups, namely, Se+/Le+, Se+/Le−, Se−/Le+, and Se−/Le−. The abundance of some specific HMOs can reflect the HMO phenotype.[20–22] In our study, the detection of both 2'-FL and LNFP I at very high intensity in the breast milk sample BM1 suggested that this sample came from a mother-group Se+ (Figure 3 and Table 1).[19,23] The presence of two additional HMOs such as LNDFH-I and LDFT in this same sample may reflect a group Se+/Le+.[21,24] Although 2'-FL was also detected in the breast milk sample BM3, its intensity was very low compared with the breast milk sample BM1 (about 30 times lower). For the two other breast milk samples, BM2 and BM4, neither 2'-FL nor LNFP I were detected, suggesting that they should belong to mothers Se−. It has been reported that the presence of LNFP II, LNFP III, and 3-FL is characteristic of the phenotype Le+ and their levels are higher in breast milk from mothers Se−/Le+ compared with breast milk from mothers Se+/Le+.[21] However, the detection of both 3-FL and LNFP-III in breast milk from mothers Se−/Le− has been stated.[24] Here, using our approach, 3-FL was observed in three of the four breast milk samples (i.e., BM1, BM2, and BM3) and no conclusions can be drawn about LNFP III as we did not have LNFP III standard. For the breast milk sample BM4, its overall HMO content was very low. Neither 2'-FL nor 3-FL nor LNFP I could be detected under our analytical conditions and only a peak corresponding to the unresolved signals between LNFP II and LNFP VI was

TABLE 1 Comparison of ion mobility–mass spectrometry (IM-MS) responses of selected human milk oligosaccharide (HMO) isomers from analysis of four breast milk samples, BM1 to BM4 using single ion mobility monitoring (SIM^2) acquisition (see Figure S3).

Sample	[2'-FL]	[3-FL]	[LDFT]	[LNDFH I]	[LNFP I]	[LNFP II]/[LNFP VI]	[LNFP V]
BM1	+++	+	++	++	+	++	Trace
BM2	-	+	-	-	-	+	Trace
BM3	+	+	Trace	Trace	Trace	+	Trace
BM4	-	-	-	-	-	++	Trace

Note: +++: high intensity; ++: medium intensity; +: low intensity; trace: very low intensity; -: not determined.
Abbreviations: 2'-FL, 2′-fucosyllactose; 3-FL, 3-fucosyllactose; LDFT, lactodifucotetraose; LNDFH I, lacto-N-difucohexaose I; LNFP I, lacto-N-fucopentaose I.

observed with the highest intensity compared with that of other breast milk. However, no separation between LNFP II and LNFP VI could be achieved here, despite considering the *m/z* 876 $[M+Na]^+$ species which offered the best distinction between the LNFP isomers compared with other species generated by negative or positive ionization mode (Figure 3). The combination with complementary MS/MS experiments is required to improve the distinction of the four isomers of LNFP.[12]

Although relative abundances of some HMO isomers could be estimated here, the use of an authentic HMO standard should provide more accurate measurements, that is, absolute quantification. Here, the potential of the SIM^2 mode for rapid quantification of an HMO isomer in a complex matrix was evaluated based on the determination of 2'-FL. This HMO was chosen as a representative isomer because it could not be separated from 3-FL in full IM detection and it is biologically relevant because it is the most abundant among the fucosyl-oligosaccharides that exhibit protective activity against several pathogens, particularly for protection against infant diarrhea.[25]

A dilution factor of 2000 was chosen to do this investigation using the direct IM-MS approach. Under the full IM detection conditions, the IM resolving power was not sufficient to obtain a good separation between 2'-FL and 3-FL, so SIM^2 acquisition was applied to improve their separation. The two IM peaks corresponding to the *m/z* 487 $[M-H]^-$ species of 2'FL were better separated but that of 3-FL was partially separated from the second peak of 2'-FL (Figure S4). Note that the detection of multiple mobility peaks as observed here for 2'FL could result from different anomeric conformers or conformations for a given species.[11] Although, there was no satisfactory separation between the second peak of 2'-FL $[M-H]^-$ species and that of 3-FL, the resolution of the first peak of 2'-FL appeared to be sufficient for its specific detection. Therefore, quantification of 2'-FL was performed by measuring the first peak area. Note that baseline IM separation between 2'-FL and 3-FL was obtained for the *m/z* 523 $[M+Cl]^-$ species but the $[M+Cl]^-$ signal could not be used to accurately quantify 2'-FL due to its poor linearity response (Figure S5).

Then, the IM-MS response of the 2'-FL standard was measured three times at each concentration level using our direct approach. Two calibration curves were built from the FIA-IM-MS measurements: (i) the first using an external standard calibration method with a series of standard solutions in solvent (i.e., water–methanol, 1:1, v/v), and (ii) the second using a standard addition method with the breast milk sample BM5 spiked at five concentration levels of 2'-FL (i.e., 0.05, 0.2, 0.5, 0.7, and 1 $ng.\mu L^{-1}$). A good response linearity was observed for both curves with R^2 of 0.97 and 0.99 for the external standard calibration and the standard addition method, respectively (Figure 4A). These two curves

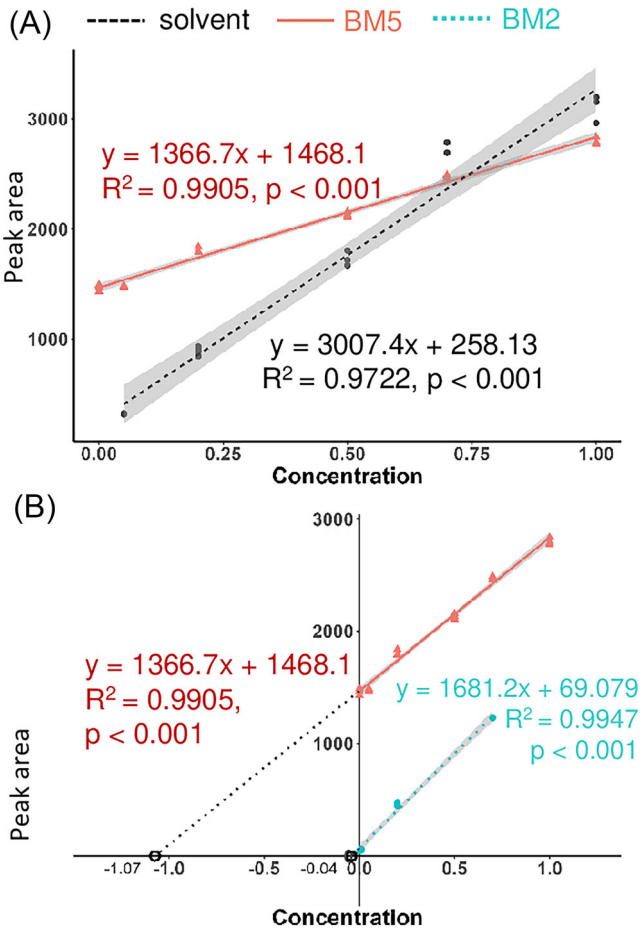

FIGURE 4 Calibration curves superimposed with 95% confidence interval for the measured ion mobility–mass spectrometry (IM-MS) response of the 2′-fucosyllactose (2'-FL) *m/z* 487 $[M-H]^-$ species from the single ion mobility monitoring (SIM^2) acquisition: using (A) standard calibration solutions prepared in water–methanol (1:1, v/v) and the breast milk sample BM5 spiked with 2'-FL standard solutions, and (B) the comparison between two standard addition method curves obtained from two spiked breast milks, BM2 and BM5.

fitted well to the standard concentration range considered with a relative standard deviation (RSD) ranging from 1.5% to 4.7% for external calibration, and RSD < 2.1% for standard addition method ($n = 3$ for each concentration level). As expected, the significant deviation from zero of the y-intercept of the standard addition method curve confirmed the presence of endogenous 2'-FL in the breast milk sample BM5 (Figure 4). However, the slopes of the two curves (i.e., slope = 3007.4 for the external standard calibration curve *vs.* slope = 1366.7 for the standard addition method) were completely different, reflecting the change in 2'-FL response between analyses performed in solvent and in the breast milk sample, with a lower 2'-FL intensity in breast milk. Most likely, this reduced detection sensitivity may be due to matrix effects caused by milk components, even if a 1:2000 dilution of the breast milk was carried out before performing the FIA-IM-MS analysis.[17] Therefore, using the solvent-based calibration curve is expected to lead to an underestimated amount of 2'-FL. Indeed, a 2'-FL concentration of 0.40 ng.µL^{-1} was obtained for the 1:2000 diluted breast milk sample BM5 using the equation of the solvent-based calibration curve while that provided by the standard addition curve, corresponding to the absolute value of the x-intercept, was 1.07 ng.µL^{-1} (Figure 4B). This difference corresponding to a bias of 67% in the determination of the 2'-FL concentration clearly underlined that the solvent-based calibration curve could not be used for the determination of 2'-FL in breast milk samples because it did not take into account the still-existing matrix effects. However, performing a standard addition method for every sample is a long and tedious process (including different sample preparation steps and the long analysis time), particularly for a large number of samples. Nevertheless, this approach is of great use in overcoming any matrix effects without using an internal standard, especially in the absence of a compatible blank matrix.

The repeatability and accuracy criteria were evaluated on the breast milk sample BM5 spiked with 2'-FL at two concentration levels: (i) a low level at 0.05 ng.µL^{-1} ($n = 5$) and (ii) a middle level at 0.2 ng.µL^{-1} ($n = 6$). A bootstrap procedure ($p = 100$) was applied to achieve a more robust prediction of these two concentration levels. The calibration curves were therefore calculated using randomly selected data from IM-MS measurements of the breast milk sample BM5 spiked with different concentration levels of 2'-FL: three measurements among the five repetitions for the low concentration (i.e., 0.05 ng.µL^{-1}), three measurements among the six repetitions for the middle concentration (i.e., 0.2 ng.µL^{-1}); and to get more variable conditions one measurement for the other concentrations were randomly removed for the regression calculations. RSD values of 35.3% and 6.0% were obtained for low and middle concentrations, respectively. An acceptable recovery of 125.5% for 2'-FL was obtained for the spiked level at 0.2 ng.µL^{-1}, while a lower recovery of 58.5% was found for the concentration at 0.05 ng.µL^{-1}, suggesting that the limit of quantification should be slightly higher.

The standard addition method was also applied to another breast milk sample, BM2, which was spiked with three concentrations of 2'-FL (i.e., 0.01, 0.2, and 0.7 ng.µL^{-1}) in order to evaluate matrix effects. The two calibration curves obtained from two breast milk samples BM5 and BM2 were compared (Figure 4B). The slopes of the two curves are relatively close (i.e., 1366.7 for the slope from the breast milk sample BM5, ranging from 1303 to 1430 in a 95% confidence interval, and 1681.2 for the slope from the breast milk sample BM2, ranging from 1580 to 1783 within a 95% confidence interval). This reflects fairly equivalent matrix effects in the two breast milk samples despite their different HMO compositions. Furthermore, the x-intercept of the calibration curve from the breast milk BM2 was almost zero (x = −0.04), indicating that this breast milk does not contain (or at least a very small amount of) 2'-FL and could therefore come from a non-secretor mother. So, it would be interesting to use this breast milk sample as a "blank" matrix to quantify 2'-FL in other milk samples. The determination of 2'-FL in the breast milk sample BM5 diluted to 1:2000 was carried out using the calibration curve obtained from the breast milk BM2. The obtained value of 0.83 ng.µL^{-1} value differed by 22% compared with the concentration of 1.07 ng.µL^{-1} extrapolated from the calibration curve using breast milk BM5, which is quite satisfactory.

To simplify the quantification procedure, the standard addition method can also be performed with a single point by preparing only one standard addition sample.[26] The concentration of the analyte present in the unknown sample is then determined by comparing its signal detected in the sample alone with that of the same sample spiked with a known concentration of analyte because the analyte response is proportional to its concentration. Although a single-point calibration is expected to be less robust than linear regression, such an approach allowed a more rapid determination of analyte overcoming matrix effects that can vary from sample to sample.

4 | CONCLUSION

The ability of IM to differentiate isomers and produce quantitative data using FIA mode is demonstrated here. The combination of direct introduction and IM-MS using SIM^2 acquisition allowed the separation and distinction of some specific HMOs, highlighting different HMO profiles according to maternal phenotypes. Furthermore, we presented a simple and rapid approach for the determination of 2'-FL without any prior chemical derivatization, as a proof of concept for assaying isomer compounds in a complex matrix. This study demonstrated that adequate sample dilution is essential in any quantification, thereby assessing the proportionality of the analyte response related to matrix effects. The validity of the standard addition method was proven by its good linearity, repeatability, and robustness. With single-point calibration, our FIA-IM-MS approach will be very useful for high-throughput quantification. Its application will be also beneficial in any analytical field where isomer determination is required.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was carried out in accordance with the ethical standards of the National Research Committee and the 1964 Helsinki Declaration and its later amendments. Informed consents were obtained from all mothers included in the previous study[13] and all the samples were anonymized.

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