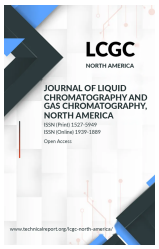

Journal of Liquid Chromatography and Gas Chromatography, North America
https://technicalreport.org/lcgcnam

LC GC N. Am., Vol. 42, No.1, 14-20, 2024

Technical Reports Press

RESEARCH ARTICLE

Diagnostic-Fragment-Gated Reporter-Ion Normalization for Quantitative Mapping of Glycerophospholipid *sn*-Positional Isomers

Louis E. Brus[1*]

[1]*Department of Chemistry, Columbia University, New York, New York 10027, USA*
[*]*Corresponding author*

Corresponding author:
leb26@columbia.edu

Received: 23 November 2025
Accepted: 23 November 2025
Available online: 23 November 2025

Abstract

Isomer-resolved lipidomics needs a direct analytical connection between structural assignment and relative abundance. Summed-composition names can merge distinct phosphatidylcholine, phosphatidylethanolamine, and phosphatidylglycerol arrangements into one lipid entry even when fatty-acyl placement on glycerol carries biochemical significance. This work establishes a diagnostic-fragment-gated version of Diagnostic Reporter-Ion Normalization (DRIN) for multistage mass spectrometry, in which quantitative ratios are accepted only when reporter ions arise from an assigned *sn*-positional fragment. The central question is whether reporter-ion measurements can be converted into calibrated and chemically interpretable lipid-isomer maps while keeping response linearity, matrix transfer, and plasma directionality as separate analytical claims. The measurement set comprises PC 18:1/16:0 calibration values, a two-concentration *Escherichia coli* lipid-extract comparison, and a human plasma phosphatidylcholine isomer profile. PC 18:1/16:0 produced near-proportional reporter response at both lipid and *sn*-isomer levels, with slopes of 1.0906 and 1.1274. In the bacterial extract comparison, 15 assigned PG and PE isomers recovered the intended two-fold relation with 87.0–97.5% accuracy. In plasma, PC 18:0/18:2, PC 16:0/18:2, PC 18:1/16:0, and PC 18:0/18:1 showed the strongest enrichment, whereas PC 14:0/20:4, PC 16:0/20:5, and PC 18:0/22:6 showed pronounced depletion. These findings answer the analytical question by showing that reporter-ion ratios become defensible isomer ratios only when diagnostic-fragment identity, calibration behavior, and matrix compatibility are evaluated together.

Keywords: analytical lipidomics; glycerophospholipid; *sn*-positional isomer; isobaric mass tag; diagnostic reporter ion; MS3; plasma phosphatidylcholine; *Escherichia coli*

1. Introduction

Glycerophospholipids participate in membrane architecture, vesicle trafficking, lipid-protein organization, and enzyme-mediated remodeling. Their names are often compressed into head group, total carbon number, and unsaturation count, but that convention does not define the complete molecular arrangement. The placement of fatty acyl chains at the *sn*-1 and *sn*-2 positions can reflect phospholipase activity, Lands-cycle reacylation, fatty-acid availability, and tissue-selective remodeling. This positional dimension is particularly relevant for phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), and phosphatidylglycerols (PGs), where one summed composition may correspond to multiple molecular structures with different biochemical implications [1–3].

Summed-composition lipidomics provides useful coverage but suppresses structural directionality. A shorthand such as PC 36:2 communicates class and aggregate composition while concealing entries such as PC 18:0/18:2 and PC 18:1/18:1. The distinction is not merely nomenclatural. Acyl-chain placement influences bilayer packing, enzymatic accessibility, and interpretation of lipid remodeling in disease-associated material. Standardized lipid nomenclature has improved communication across laboratories, yet a comparative conclusion remains reliable only when the structural evidence supports the level of molecular detail being claimed [4–6].

Cite as: L. E. Brus (2024). Diagnostic-Fragment-Gated Reporter-Ion Normalization for Quantitative Mapping of Glycerophospholipid *sn*-Positional Isomers. LC GC N. Am., 42(1) (2024) 14-20.

This work is licensed under Creative Commons Attribution-NonCommercial 4.0 International License

Mass spectrometry offers several routes for deeper lipid characterization. Electrospray ionization and tandem mass spectrometry can establish head group and acyl-chain evidence for many glycerophospholipids, while multistage activation improves selectivity by isolating product ions before subsequent fragmentation. Collision-induced dissociation remains a core tool, but *sn*-position and carbon-carbon double-bond assignments often need additional gas-phase chemistry or selective ion reactions to generate diagnostic fragments [7–9].

Advanced lipid-isomer methods have expanded this capability. Ozone-induced dissociation provides predictable fragments for double-bond localization; Paterno–Buchi photochemistry supports identification and quantification of carbon-carbon double-bond location isomers; epoxidation, electrochemical reactions, and metal-assisted chemistry provide complementary structural information [10–12]. Ion/ion charge inversion and high-coverage structural lipidomics have further demonstrated that several isomer dimensions can be accessed in complex mixtures when the fragmentation chemistry is selective enough [13–15].

Identification and quantification, however, are different analytical achievements. A diagnostic fragment may establish that a positional arrangement is present, but comparative lipidomics also requires a defensible ratio for that same arrangement. Isobaric reporter-ion strategies are attractive because tagged samples can be combined and measured under shared instrumental conditions. The important restriction is that a reporter-ion ratio does not automatically represent an isomer ratio. It becomes one only when both reporter ions are released through a structurally assigned diagnostic channel [16–18].

This restriction is essential because structurally related lipids can co-exist within the same precursor selection window. A reporter signal measured without diagnostic gating may reflect a pooled precursor, a co-isolated ion, or an insufficiently specific structural channel. The analytical task is therefore to decide when a numerical ratio has enough chemical support to be interpreted as a ratio for a particular *sn*-positional isomer. Calibration response, fragment identity, and matrix behavior must be judged as connected parts of the same measurement logic rather than as disconnected observations [19–21].

The research question is whether multistage reporter-ion measurements can be converted into calibrated and chemically interpretable maps of glycerophospholipid *sn*-positional perturbation while preserving the link between diagnostic fragments and reporter-ion ratios. The question is analytical in scope. It asks whether response behavior in a PC standard, quantitative transfer in a bacterial lipid extract, and directionality in a plasma PC profile can be separated and then recombined into a coherent isomer-level interpretation.

DRIN is formulated here as a diagnostic-fragment-gated calculation. The *sn*-diagnostic fragment is treated as the quantitative entry point; calibration inversion is applied where response terms are available; class transfer is restrained when calibration coverage is incomplete; and each lipid is assigned a directional class only after structural compatibility has been considered. This approach differs from a simple abundance table because it does not allow the reporter ratio to stand alone without molecular evidence.

The analytical record contains three connected layers. The first evaluates PC 18:1/16:0 calibration at the lipid and *sn*-isomer levels. The second assesses recovery of a known two-fold relation for PG and PE isomers in *E. coli* lipid extracts. The third interprets human plasma PC isomer ratios by separating enriched, depleted, and near-neutral structures. Together, these layers test whether diagnostic reporter ions can support calibrated positional-isomer mapping rather than only class-level lipid comparison.

This orientation places the work within analytical chemistry, spectroscopy, instrumentation, and structural mass spectrometry. The plasma measurements are used as a demanding chemical application rather than as a stand-alone clinical validation. The aim is to define when *sn*-positional reporter-ion evidence is strong enough to support interpretable comparative lipid signatures.

2. Materials and Methods

2.1. Measurement record and analytical organization

The numerical inputs come from the aziridine-based isobaric mass-tagging study of unsaturated lipid *sn*-positional isomers by Yang, Tang, Feng, and Yan [22]. The values used here are the PC 18:1/16:0 calibration response, the two-concentration *E. coli* lipid-extract comparison, the human plasma PC ratio profile, and the associated PC isomer assignments. The citation identifies the experimental origin of the measurements, while the present calculation organizes those measurements into a diagnostic-fragment-gated interpretation.

The measurements were divided into three analytical layers. The calibration layer tests whether reporter-ion response remains proportional to prepared concentration ratios. The bacterial matrix layer tests whether assigned diagnostic-fragment reporter ions recover a known two-fold relation in a chemically crowded extract. The plasma layer tests whether the calculation yields molecular directionality for PC *sn*-positional isomers in cancer and healthy plasma. Keeping these layers separate prevents the plasma comparison from carrying more interpretive weight than the calibration and matrix evidence allow.

The organization in Table 1 defines the evidentiary sequence. Calibration determines whether the reporter signal can be treated quantitatively. The bacterial extract assesses whether that behavior survives lipid-class and matrix complexity. The plasma profile is interpreted only after these two checks have established that the signal has a defensible chemical basis.

2.2. Diagnostic-fragment-gated DRIN calculation

For lipid isomer i, the local-background-corrected reporter-ion ratio is calculated as

$$R_i = \frac{I_{114,i} - B_{114,i}}{I_{117,i} - B_{117,i}}, \tag{1}$$

where $I_{114,i}$ and $I_{117,i}$ denote the reporter-ion intensities assigned to the same *sn*-diagnostic fragment, and $B_{114,i}$ and $B_{117,i}$ denote local background estimates. This expression is only the numerical starting point. It is interpreted as lipid-isomer evidence only when both reporter ions are tied to the assigned diagnostic fragment.

Calibration correction is obtained by inversion of the response equation:

$$\widehat{\rho}_i = \frac{R_i - \beta_c}{\alpha_c}, \tag{2}$$

where α_c and β_c are the response slope and intercept for class or experiment type c. This step separates a measured reporter ratio from an estimated concentration ratio. When class-specific calibration is unavailable, the ratio is treated cautiously instead of being given unwarranted numerical certainty.

For lipid classes without complete calibration coverage, the calculation uses a restrained class-transfer estimate:

$$\widehat{\rho}_{i,\lambda} = \lambda\widehat{\rho}_i + (1-\lambda)R_i, \qquad 0 \le \lambda \le 1. \tag{3}$$

The shrinkage term limits excessive transfer of the PC response curve to PE and PG entries. Values of λ closer to one emphasize calibration correction, whereas values closer to zero leave the observed reporter ratio largely unchanged. The purpose is to keep structural evidence and quantitative correction in balance when class-matched calibration is incomplete.

Diagnostic integrity is recorded through an evidence gate:

$$Q_i = \frac{S_{\mathrm{sn},i} + S_{\mathrm{acyl},i} + S_{\mathrm{rep},i}}{3}, \tag{4}$$

Table 1: Measurement layers.

Layer	Lipid evidence	Analytical question	Output used in DRIN
Calibration	PC 18:1/16:0 ratio series	Does reporter response remain proportional?	Slope, intercept, and predicted ratio
Bacterial matrix	Assigned PG and PE isomers in two *E. coli* levels	Does diagnostic reporting hold in a mixed extract?	Recovery of the two-fold relation
Plasma directionality	Human PC *sn*-positional ratios	Which structures move upward or downward?	Enriched, depleted, and near-neutral entries

where $S_{\mathrm{sn},i}$ denotes the expected *sn*-diagnostic fragment, $S_{\mathrm{acyl},i}$ denotes consistency of acyl-chain evidence, and $S_{\mathrm{rep},i}$ denotes measurable reporter ions above local noise. The value is used as an acceptance gate, not as an ornamental confidence tag. A lipid with weak structural support is therefore not ranked in the same way as a structurally coherent primary entry.
The final direction score is

$$D_i = Q_i \log_2(\widehat{\rho}_{i,\lambda}). \tag{5}$$

Positive values indicate enrichment in the cancer-associated channel, negative values indicate depletion, and values near zero indicate limited separation. This expression prevents magnitude alone from controlling interpretation. A large ratio without diagnostic integrity is not promoted to a leading entry, whereas a moderate ratio with consistent fragment evidence can remain analytically meaningful.

2.3. Calibration and classification criteria

The PC 18:1/16:0 calibration series used prepared ratios of 1, 2, 5, and 10. Lipid-level reporter response and *sn*-isomer-level reporter response were both considered. The lipid-level response reflects the tagged precursor channel, whereas the *sn*-isomer-level response reflects reporter ions released after diagnostic-fragment selection. DRIN uses the latter response as the primary quantitative route for positional-isomer interpretation.
Directional classes were assigned from the cancer-to-healthy ratio in the plasma profile. Ratios of at least 2.0 were classified as enriched, ratios of 0.5 or lower were classified as depleted, and ratios between 0.5 and 2.0 were treated as intermediate or near-neutral according to log-scale displacement. These categories are analytical ranking rules for candidate isomers, not clinical decision thresholds.

3. Results and Discussion

3.1. Structural specificity before quantification

The first requirement is to distinguish a summed lipid name from a diagnostic-fragment-supported isomer entry. Conventional notation remains useful, but it does not specify which fatty acyl chain occupies the *sn*-1 or *sn*-2 position. A reporter-ion ratio attached only to a summed name can therefore behave as a bulk precursor ratio rather than as a positional-isomer ratio.

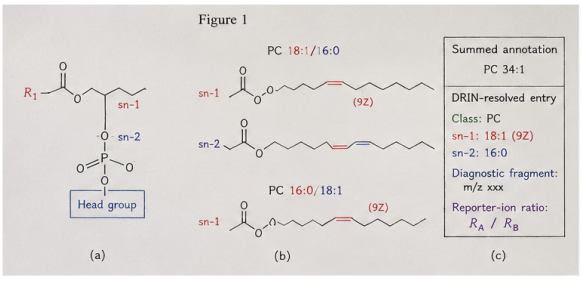

Figure 1: DRIN structural gate.

The example in Figure 1 shows why the calculation begins with molecular structure rather than intensity alone. One summed annotation can contain alternative positional arrangements, whereas the DRIN entry keeps lipid class, acyl placement, diagnostic fragment, and reporter relation together. The resulting interpretation is that quantification and assignment are inseparable: without the diagnostic fragment, the reporter ratio lacks sufficient chemical specificity for a positional-isomer conclusion.

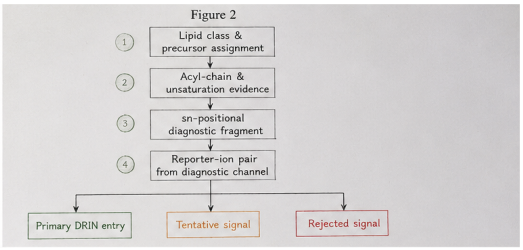

Figure 2: DRIN acceptance route.

The sequence in Figure 2 converts this structural requirement into an acceptance route. Precursor and class evidence are checked first, followed by acyl-chain evidence, *sn*-diagnostic fragment formation, and paired reporter-ion measurement. This order prevents a numerical ratio from entering the interpretation before the ion evidence has been evaluated. Entries that fail the sequence remain useful for chemistry optimization, but they are not assigned the same analytical status as accepted DRIN entries.

3.2. Calibration response and quantitative meaning

The calibration record supports the quantitative premise of the method. PC 18:1/16:0 produced a lipid-level response slope of 1.0906 and an *sn*-isomer-level slope of 1.1274, with coefficients of determination close to unity. The difference between these slopes indicates that diagnostic-fragment reporter ions preserve proportional response but are not identical to the precursor-level channel. Calibration is therefore a necessary part of the interpretation rather than a cosmetic adjustment.

Table 2: Calibration terms.

Quantitative layer	Reporter-ion origin	Response equation	R^2
Lipid-level response	Tagged lipid precursor	$y = 1.0906x + 0.0072$	≈ 0.9999
sn-isomer response	*sn*-diagnostic fragment	$y = 1.1274x - 0.1661$	≈ 0.9990

x denotes the prepared concentration ratio and y denotes the measured reporter-ion ratio.

The response terms in Table 2 show that the diagnostic-fragment reporter channel is quantitatively usable but should not be treated as interchangeable with the precursor-level channel. The negative intercept in the *sn*-isomer equation slightly depresses response near unity and contributes to stronger correction at higher ratios. This matters for plasma interpretation because the largest positive and negative PC changes should be ranked after response behavior has been considered, not from raw ratios alone.
The calibration behavior in Figure 3 confirms that both response layers follow the prepared concentration series. The analytical significance is that multistage selection does not eliminate concentration information from the *sn*-diagnostic channel. This result supports the central use of

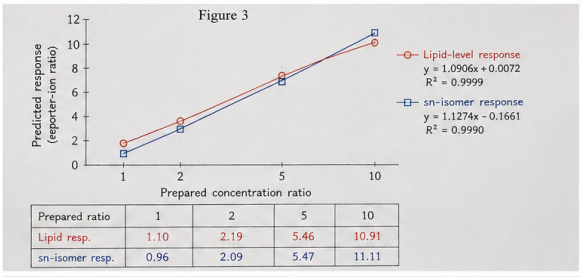

Figure 3: Reporter calibration.

DRIN: positional assignment and relative quantification can be coupled when reporter ions originate from the diagnostic fragment itself.

Table 3: Predicted ratios.

Prepared ratio	Lipid predicted	*sn*-isomer predicted	*sn*deviation	Analytical use
1	1.10	0.96	-0.04	Equal-mixture check
2	2.19	2.09	+0.09	Low-ratio response
5	5.46	5.47	+0.47	Mid-range evaluation
10	10.91	11.11	+1.11	Upper-range correction

The predicted values in Table 3 make the calibration effect visible. At low ratio, the diagnostic-fragment response remains close to the prepared value. At the upper end, the predicted *sn*-isomer response exceeds the prepared ratio, which supports calibration-aware correction before ranking highly displaced plasma entries. The practical implication is that large ratio changes should be interpreted with response terms rather than with uncorrected intensity ratios alone.

3.3. Matrix verification in *E. coli* lipid extracts

The bacterial extract comparison evaluates whether diagnostic-fragment reporter quantification remains useful in a chemically complex mixture. Unlike a single PC standard, the *E. coli* extract contains multiple PG and PE species, diverse acyl-chain combinations, and ion-chemistry features that can alter derivatization or fragmentation. The known two-fold concentration relation between the extracts provides a direct test of quantitative recovery.

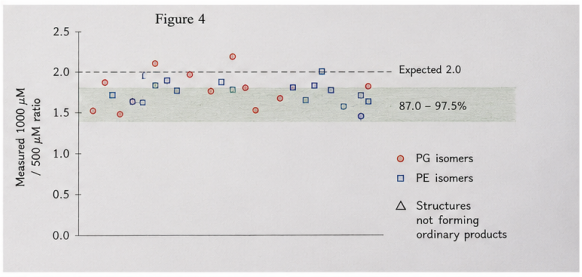

Figure 4: Bacterial recovery.

The PG and PE entries in Figure 4 cluster near the expected two-fold relation, with 87.0–97.5% recovery across 15 assigned isomers. The range indicates that matrix complexity introduces dispersion but does not abolish quantitative utility. Structures that do not form the required diagnostic products are treated separately, which reinforces the principle that lipid chemistry determines whether a reporter ratio can be accepted as an isomer ratio.

The evidence in Table 4 separates four analytical issues: prepared concentration, number of assigned isomers, recovery accuracy, and chemical exclusion. The outcome supports DRIN for lipids that pass diagnostic compatibility, but it does not imply universal transfer to every PG or PE structure. This limited claim is important because a mixed extract can confirm practical usefulness without replacing the need for broader class-specific calibration.

3.4. Human plasma PC isomer directionality

The plasma profile applies the same logic to a disease-associated comparison while remaining analytical in interpretation. The aim is not to claim clinical validity from a single molecular profile. The aim is to determine whether diagnostic-fragment reporter-ion evidence can identify which PC *sn*-positional arrangements are enriched, depleted, or near neutral when all entries belong to the same lipid class.

The values in Table 5 show that the PC class does not move as a single unit. Strong positive displacement is concentrated in selected 18:2- and 18:1-containing arrangements paired with saturated chains, whereas strong negative displacement occurs among several highly unsaturated structures. A class-level PC ratio would hide this internal redistribution. DRIN therefore changes the interpretive unit from lipid class to diagnostic-fragment-supported molecular arrangement.

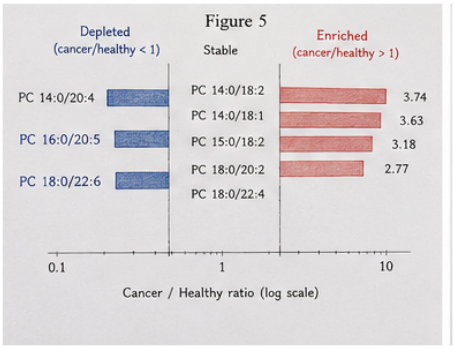

Figure 5: Plasma direction map.

The log-scale display in Figure 5 clarifies separation between enriched and depleted entries. PC 14:0/20:4 and PC 16:0/20:5 lie far below unity, whereas PC 18:0/18:2, PC 16:0/18:2, PC 18:1/16:0, and PC 18:0/18:1 exceed the enrichment threshold. The profile supports a chemically selective interpretation: the plasma difference is a redistribution among particular *sn*-positional structures, not a uniform increase or decrease in phosphatidylcholine abundance.

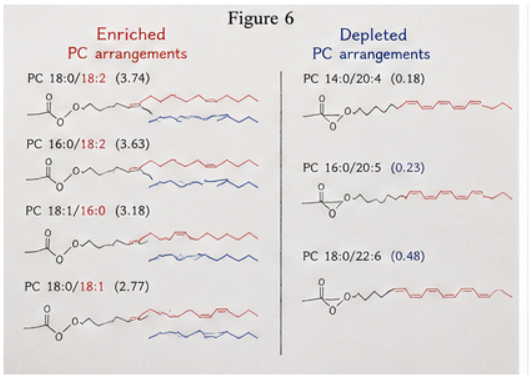

Figure 6: Dominant PC shifts.

The structural grouping in Figure 6 gives chemical context to the ratio pattern. The enriched group is dominated by saturated/unsaturated pairings containing stearoyl, palmitoyl, oleoyl, or linoleoyl chains.

Table 4: Bacterial recovery terms.

Evidence component	Observation	Analytical role	DRIN interpretation
Concentration relation	1000 μM versus 500 μM extract	Known two-fold target	Direct matrix check
Assigned isomer set	15 PG and PE *sn*-positional isomers	Mixed-class challenge	Diagnostic reporting remains usable
Accuracy interval	87.0–97.5% recovery	Quantitative tolerance	Ratios recover the intended relation
Chemical exclusion	Some 33:1 species lacked ordinary diagnostic products	Specificity control	Structural compatibility is required

Table 5: Plasma PC directionality.

PC isomer	C/H ratio	$\log_2$ ratio	Direction	Analytical interpretation
PC 14:0/18:2	0.92	-0.12	Near-neutral	Limited directional separation
PC 16:0/16:1	0.45	-1.15	Depleted	Lower monounsaturated PC signal
PC 14:0/18:1	0.86	-0.22	Near-neutral	Minor negative displacement
PC 15:0/18:2	1.24	0.31	Mildly enriched	Weak positive displacement
PC 14:0/20:4	0.18	-2.47	Depleted	Strong arachidonate-containing loss
PC 16:0/18:2	3.63	1.86	Enriched	Strong linoleate-containing gain
PC 18:1/16:0	3.18	1.67	Enriched	Increased reversed acyl placement
PC 16:0/20:5	0.23	-2.12	Depleted	Marked highly unsaturated PC loss
PC 16:0/20:4	0.67	-0.58	Depleted	Moderate negative displacement
PC 16:0/20:3	0.76	-0.40	Depleted	Moderate negative displacement
PC 18:0/18:2	3.74	1.90	Enriched	Largest positive PC response
PC 18:0/18:1	2.77	1.47	Enriched	Strong stearoyl/oleoyl gain
PC 16:0/22:6	1.68	0.75	Enriched	Intermediate docosahexaenoyl-containing gain
PC 18:0/20:2	1.32	0.40	Mildly enriched	Limited positive displacement
PC 18:0/22:6	0.48	-1.06	Depleted	Lower docosahexaenoyl PC signal
PC 18:0/20:5	0.62	-0.69	Depleted	Moderate negative displacement
PC 18:0/22:4	0.91	-0.14	Near-neutral	Limited directional separation

The depleted group includes arachidonate-, eicosapentaenoyl-, and docosahexaenoyl-containing PC entries. The finding argues against treating highly unsaturated PCs as one uniform category, because several decrease strongly while selected 18:1 and 18:2 arrangements increase.

The grouping in Table 6 converts the long plasma list into targeted follow-up logic. Strongly enriched and strongly depleted entries are separated from intermediate and near-neutral structures, allowing future targeted acquisition to focus on the highest-yield candidates. The table deliberately avoids biomarker claims: it supports prioritization of candidate *sn*-positional isomers for replication, not immediate diagnostic deployment.

3.5. Interpretive advantage over summed-composition lipidomics

The central advantage of DRIN is the change in reporting unit. Summed-composition lipidomics reports a pooled molecular name, while DRIN reports a diagnostic-fragment-supported positional isomer together with its direction of change. This is not an annotation embellishment; it changes the type of inference that can be drawn from the mass-spectrometric measurement.

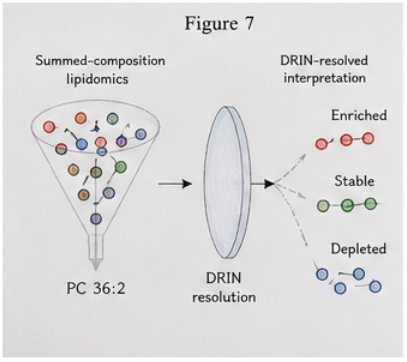

Figure 7: Isomer-level separation.

The contrast in Figure 7 illustrates how one pooled annotation can divide into enriched, depleted, and near-neutral structures once diagnostic-ion evidence is applied. This explains why DRIN can reveal opposite trends within a single lipid class. A summed PC profile might suggest broad class-level change, whereas the diagnostic-fragment-gated view shows that direction depends on acyl-chain composition and positional arrangement.

The full analytical sequence links calibration, matrix recovery, and plasma directionality. Calibration establishes proportional response. The *E. coli* comparison shows that reporter-ion behavior remains useful in a mixed extract. The plasma profile then demonstrates chemically interpretable directionality. This order prevents biological interpretation from being separated from measurement validity.

3.6. Analytical readiness and limitations

The strongest support is for PC calibration and PC *sn*-positional interpretation because the calibration record includes both lipid-level and *sn*-isomer-level response terms. The bacterial extract comparison supports extension to PG and PE entries, but broader class-specific calibration is still required. Low-abundance isomers also need caution because multistage selection can reduce signal intensity, and weak reporter pairs may be more sensitive to local noise or co-isolated ions.

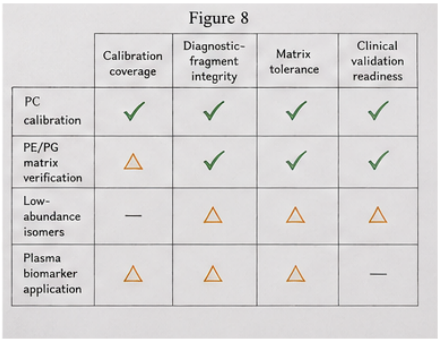

Figure 8: Analytical readiness.

The readiness map in Figure 8 presents the method with defined boundaries. PC calibration has the strongest support; PE and PG matrix recovery is promising but requires wider calibration; low-abundance isomers need targeted confirmation; and plasma application requires independent cohort-scale validation before clinical language is appropriate. The method therefore answers the research question for calibrated diagnostic-fragment interpretation while identifying the measurement conditions that still require additional validation.

Future analytical work should add class-specific calibration panels, replicate diagnostic-fragment integrity scoring, and orthogonal confirmation by targeted MS/MS, ion mobility, OzID, Paterno–Buchi chemistry, or charge-inversion approaches. Larger plasma cohorts are necessary before diagnostic claims can be made. The immediate next step is controlled evaluation of reporter-ion response across lipid class, chain length, unsaturation, and matrix composition.

Table 6: Plasma priority groups.

Priority group	PC entries	Analytical meaning
Strong enrichment	PC 18:0/18:2; PC 16:0/18:2; PC 18:1/16:0; PC 18:0/18:1	Leading candidates for targeted confirmation because ratios exceed the enrichment threshold
Strong depletion	PC 14:0/20:4; PC 16:0/20:5; PC 18:0/22:6	Leading negative signals involving highly unsaturated chains
Intermediate displacement	PC 16:0/22:6; PC 18:0/20:2; PC 15:0/18:2; PC 16:0/20:4; PC 16:0/20:3; PC 18:0/20:5	Directionally useful entries requiring replicate confirmation before prioritization
Near-neutral response	PC 14:0/18:2; PC 14:0/18:1; PC 18:0/22:4	Limited separation under the measured comparison

4. Conclusion

This study asked whether multistage reporter-ion measurements can be converted into calibrated and chemically interpretable maps of glycerophospholipid *sn*-positional perturbation without relying on summed-composition ratios alone. The answer is affirmative within the analytical boundaries of the measurement set. DRIN succeeds because it treats the *sn*-diagnostic fragment as the quantitative entry point, requires reporter ions to originate from that structural channel, applies calibration terms where they exist, and separates primary entries from structurally weaker signals.

The calibration layer answers the response-linearity part of the question. PC 18:1/16:0 gave near-proportional response at both lipid and *sn*-isomer levels, showing that diagnostic-fragment reporter ions can preserve concentration information after multistage selection. The *E. coli* layer answers the matrix-transfer part of the question. Fifteen assigned PG and PE isomers recovered the intended two-fold concentration relation with 87.0–97.5% accuracy, showing that diagnostic reporting can remain quantitative in a complex extract when structural compatibility is satisfied. The plasma layer answers the directionality part of the question. It identifies selective redistribution of PC positional isomers, with enrichment of PC 18:0/18:2, PC 16:0/18:2, PC 18:1/16:0, and PC 18:0/18:1, and depletion of PC 14:0/20:4, PC 16:0/20:5, and PC 18:0/22:6.

The main contribution is a disciplined analytical rule: a reporter-ion ratio should be interpreted as an isomer ratio only when diagnostic-fragment identity, calibration behavior, and matrix compatibility support that interpretation. This rule creates a defensible path from multistage spectra to comparative lipid-isomer signatures and provides a focused set of *sn*-positional PC candidates for targeted replication.

Data Availability

All numerical values used for interpretation are summarized in the tables. The experimental origin of those values is identified in the Materials and Methods section.

Conflict of Interest Statement

The author declares no competing financial interest.

References

[1] van Meer, G., Voelker, D. R., & Feigenson, G. W. (2008). Membrane lipids: Where they are and how they behave. *Nature Reviews Molecular Cell Biology, 9*(2), 112–124.

[2] Robichaud, P. P., Boulay, K., Munganyiki, J.-E., & Surette, M. E. (2013). Fatty acid remodeling in cellular glycerophospholipids following the activation of human T cells. *Journal of Lipid Research, 54*(10), 2665–2677.

[3] Lands, W. E. (2000). Stories about acyl chains. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1483*(1), 1–14.

[4] Liebisch, G., Fahy, E., Aoki, J., Dennis, E. A., Durand, T., Ejsing, C. S., . . . & Spener, F. (2020). Update on LIPID MAPS classification, nomenclature, and shorthand notation for MS-derived lipid structures. *Journal of Lipid Research, 61*(12), 1539–1555.

[5] Rustam, Y. H., & Reid, G. E. (2018). Analytical challenges and recent advances in mass spectrometry-based lipidomics. *Analytical Chemistry, 90*(1), 374–397.

[6] Holčapek, M., Liebisch, G., & Ekroos, K. (2018). Lipidomic analysis.

[7] Pulfer, M., & Murphy, R. C. (2003). Electrospray mass spectrometry of phospholipids. *Mass Spectrometry Reviews, 22*(5), 332–364.

[8] Murphy, R. C., & Axelsen, P. H. (2011). Mass spectrometric analysis of long-chain lipids. *Mass Spectrometry Reviews, 30*(4), 579–599.

[9] Han, X., & Gross, R. W. (2003). Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: A bridge to lipidomics. *Journal of Lipid Research, 44*(6), 1071–1079.

[10] Thomas, M. C., Mitchell, T. W., Harman, D. G., Deeley, J. M., Nealon, J. R., & Blanksby, S. J. (2008). Ozone-induced dissociation: Elucidation of double bond position within mass-selected lipid ions. *Analytical Chemistry, 80*(1), 303–311.

[11] Ma, X., Chong, L., Tian, R., Shi, R., Hu, T. Y., Ouyang, Z., & Xia, Y. (2016). Identification and quantitation of lipid C=C location isomers: A shotgun lipidomics approach enabled by photochemical reaction. *Proceedings of the National Academy of Sciences, 113*(10), 2573–2578.

[12] Feng, Y., Chen, B., Yu, Q., & Li, L. (2019). Identification of double bond position isomers in unsaturated lipids by m-CPBA epoxidation and mass spectrometry fragmentation. *Analytical Chemistry, 91*(3), 1791–1795.

[13] Randolph, C. E., Blanksby, S. J., & McLuckey, S. A. (2019). Toward complete structure elucidation of glycerophospholipids in the gas phase through charge inversion ion/ion chemistry. *Analytical Chemistry, 92*(1), 1219–1227.

[14] Zhao, X., Zhang, W., Zhang, D., Liu, X., Cao, W., Chen, Q., . . . & Xia, Y. (2019). A lipidomic workflow capable of resolving *sn*- and C=C location isomers of phosphatidylcholines. *Chemical Science, 10*(46), 10740–10748.

[15] Cao, W., Cheng, S., Yang, J., Feng, J., Zhang, W., Li, Z., . . . & Ma, X. (2020). Large-scale lipid analysis with C=C location and *sn*-position isomer resolving power. *Nature Communications, 11*(1), 375.

[16] Yang, T., Tang, S., Kuo, S. T., Freitas, D., Edwards, M., Wang, H., . . . & Yan, X. (2022). Lipid mass tags via aziridination for probing unsaturated lipid isomers and accurate relative quantification. *Angewandte Chemie, 134*(39), e202207098.

[17] Tang, S., Chen, X., Ke, Y., Wang, F., & Yan, X. (2022). Voltage-controlled divergent cascade of electrochemical reactions for characterization of lipids at multiple isomer levels using mass spectrometry. *Analytical Chemistry, 94*(37), 12750–12756.

[18] Chen, X., Tang, S., Freitas, D., Hirtzel, E., Cheng, H., & Yan, X. (2022). Characterization of glycerophospholipids at multiple isomer levels via Mn(II)-catalyzed epoxidation. *Analyst, 147*(21), 4838–4844.

[19] Cajka, T., & Fiehn, O. (2016). Toward merging untargeted and targeted methods in mass spectrometry-based metabolomics and lipidomics. *Analytical Chemistry, 88*(1), 524–545.

[20] Zhang, W., Jian, R., Zhao, J., Liu, Y., & Xia, Y. (2022). Deep-lipidotyping by mass spectrometry: Recent technical advances and applications. *Journal of Lipid Research, 63*(7), 100219.

[21] Grooms, A. J., Nordmann, A. N., & Badu-Tawiah, A. K. (2022). Plasma-droplet reaction systems: A direct mass spectrometry approach for enhanced characterization of lipids at multiple isomer levels. *ACS Measurement Science Au, 3*(1), 32–44.

[22] Yang, T., Tang, S., Feng, J., & Yan, X. (2024). Lipid isobaric mass tagging for enhanced relative quantification of unsaturated *sn*-positional isomers. *ACS Measurement Science Au, 4*(2), 213–222.