

LICAR Manual

Version 1.0

L. Gao, S. Ji, B. Burla, M.R. Wenk, F. Torta and A. Cazenave-Gassiot

October 26, 2020

This document constitutes a brief introduction to using SLING's Lipids Isotopic Correction Application in R (LICAR). LICAR is a Shiny application that takes *.csv* (comma separated values) files containing targeted lipidomics (*i.e.* peak abundance data, such as areas under the curve of lipid species in a batch of samples analysed by MRM) and applies isotopic correction based on the used MRM patterns. Note that this is only applicable when lipid species within a given lipid class have been analysed by *MRM* and have *not* been chromatographically separated.

1. Prepare the *.csv* files

1.1. Separate data based on lipid classes and MRM patterns

Peak integration data should be prepared in separate *.csv* files for each lipid class and MRM pattern. For example, if PE were measured both in positive ionisation using MRM transitions based on the neutral loss of 141 *and* in negative ionisation using fatty acyl-based MRM transition, then these data will be prepared in *two separate files*, as shown in Figure 1. When uploading data files to the application (see section 3.1), failure to separate lipid classes in separate files should result in the error message "Lipid class is not unique, please check the data!"

	A	B	C	D	E	F	G	H	I
1	Name	Precursor	Product	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	
2	PE 32:1	690.5	540.5	5862	7478	5372	6289	6471	
3	PE 33:2	702.5	561.5	19619	22310	20904	20387	20560	
4	PE 33:3	700.5	559.5	16422	21557	22056	16738	20718	
5	PE 34:0	720.6	579.6	10265	12008	11411	12011	10132	
6	PE 34:1	718.5	577.5	86555	91009	99769	91019	90682	
7	PE 34:2	716.5	575.5	158308	191156	134754	162465	172550	
8	PE 35:2	730.5	589.5	31425	31710	34001	34653	31550	
9	PE 35:3	728.6	587.5	54711	61234	55781	61590	60548	
10	PE 35:4	726.5	585.5	100741	92450	82941	85058	88415	
11	PE 35:5	724.5	583.5	58667	66359	63844	61691	71059	
12	PE 36:1	746.6	605.6	124798	127845	105882	115035	113357	
13	PE 36:2	744.6	603.5	502692	461599	499835	472740	420450	
14	PE 36:3	742.5	601.5	150832	146863	143021	162103	163564	
15	PE 36:4	740.5	599.5	185900	179982	208940	211773	206697	
16	PE 37:1	760.6	619.6	3494	2460	3878	3160	2979	
17	PE 37:2	758.6	617.6	4769	6087	4656	4603	4713	
18	PE 37:3	756.6	615.6	14150	13801	14599	12784	13317	
19	PE 37:4	754.5	613.5	91172	102139	83624	98026	85967	
20	PE 37:5	752.5	611.5	178470	170977	164679	191771	174044	
21	PE 37:6	750.5	609.5	108954	103782	111653	108052	118062	
22	PE 37:7	748.5	607.5	38830	40534	39228	38189	38117	
23	PE 38:1	774.6	633.6	15721	15305	14389	14024	15975	
24	PE 38:2	772.6	631.6	11848	12643	11811	12032	11397	
25	PE 38:3	770.6	629.6	99561	87653	101413	95739	102005	
26	PE 38:4	768.6	627.5	489398	518672	505524	539777	530200	
27	PE 38:5	766.5	625.5	179762	169852	174291	172387	136942	

	A	B	C	D	E	F	G	H	I
1	Name	Precursor	Product	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	
2	PE 16:0/16:1	688.5	253.2	13328	11189	10678	11042	10956	
3	PE 16:1/16:0	688.5	253.2	7943	6051	6499	6987	5580	
4	PE 16:0/18:2	714.5	279.2	3273	3216	2996	2646	2665	
5	PE 18:2/16:0	714.5	255.2	2351	1631	1604	1982	1885	
6	PE 16:1/18:1	714.5	281.2	17840	14805	14589	14179	13798	
7	PE 18:1/16:1	714.5	253.2	10110	8025	8450	8035	7092	
8	PE 16:0/18:1	716.5	281.2	67543	67521	64145	57285	46505	
9	PE 18:1/16:0	716.5	255.2	26790	21107	18764	21843	17224	
10	PE 16:0/18:0	718.5	283.2	2835	2347	2187	2154	2144	
11	PE 18:0/16:0	718.5	255.2	1685	1652	1286	1686	1640	
12	PE 15:1/20:4	722.5	303.2	43428	38781	35082	36680	30483	
13	PE 15:0/20:4	724.5	303.2	5713	6375	5396	4965	5354	
14	PE 15:1/20:1	728.5	309.3	10821	13197	9806	10271	10309	
15	PE 20:1/15:1	728.5	239.2	19	64	89	67	175	
16	PE 17:1/18:1	728.5	281.2	30607	31288	28837	25483	28293	
17	PE 18:1/17:1	728.5	267.3	6547	5351	5643	5157	4978	
18	PE 16:0/20:4	738.5	303.2	35129	37351	30001	36196	28662	
19	PE 20:4/16:0	738.5	255.2	13460	10968	11572	10355	9006	
20	PE 16:0/20:3	740.5	305.3	8498	6850	6113	6612	6865	
21	PE 20:3/16:0	740.5	255.2	3437	2899	3204	2941	3282	
22	PE 16:0/20:2	742.6	307.3	1499	1518	1076	1301	1467	
23	PE 20:2/16:0	742.6	255.2	1247	1152	991	798	991	
24	PE 18:1/18:1	742.6	281.2	120782	121045	109881	102330	118375	
25	PE 16:0/20:1	744.6	309.3	1500	1426	1102	1176	1296	
26	PE 20:1/16:0	744.6	255.2	807	676	863	655	823	
27	PE 18:0/18:1	744.6	281.2	44860	49173	37176	37799	35560	

Figure 1: Peak integration data must be separated by lipid class and MRM transition types. For example, in the case of PE, positive ionisation headgroup-based data (left) as separated from negative ionisation fatty acyl-based data (right).

1.2. General *.csv* template

As seen in Figure 1, the *.csv* files have the following structure:

- the first column lists the lipid names. Please refer to section 1.3. for important notes about lipid naming requirements.
- the second columns lists the precursor ion m/z

- the third column lists the product ion m/z
- subsequent columns contain the lipids abundance values to be corrected
- Please note that the first row is reserved for column names

Example templates for all lipid classes currently covered by LICAR are available on GitHub. Please note that:

- the MRM transitions listed are by no means exhaustive, the application will “read” any lipid species within a covered class.
- to correct a lipid species (*e.g.* PC 34:1) based on the abundance of an interfering species (*e.g.* PC 34:2), both transitions must have been measured! Accurate isotopic correction in targeted lipidomics assumes that all species that could/do contribute to isotopic interference are measured so that this interference can be calculated using this application.
- LICAR currently comes with 25 pre-set from various lipid classes commonly measured in lipidomics studies. Advanced users can easily add their own MRM transition patterns for other lipid classes to extend the lipid coverage of this application.

1.3.Lipid naming

This part is important!

As mentioned above, the first column of the *.csv* files contains the lipid species names. The algorithm “reads” those names to determine the formula of the relevant fragment used in the calculation. Therefore, the names must conform to a specific nomenclature that the algorithm understands.

- The lipid class abbreviation must follow pre-defined abbreviations. These are listed in Table 1 (*e.g.* “PC” in “PC 34:1”).
- There must be a space between the lipid class abbreviation and the carbon number (*e.g.* “PC 34:1” is acceptable, while “PC34:1” is not).
- The number of carbon and the number of unsaturations must be separated by a colon, as per accepted lipid nomenclature (*e.g.* “PC 34:1”, “PC 16:0/18:1”).
- For fatty acyl-based transitions in glycerophospholipids, the fatty acyl chains can be separated by either a “/” or a “_” (*e.g.* both “PC 16:0/18:1” and “PC 16:0_18:1” are acceptable). Do note, however, that **the order in which the fatty acids are listed defines which transition is used**. In the default settings, transitions are defined by the fatty acyl chain **after** the separator. For example, measuring PC 16:0/18:1 with fatty-acyl based MRMs will make use two transitions: 804.6 -> 255.2 (for FA 16:0) and 804.6 -> 281.2 (for 18:1). In this case, the algorithm will read “PC 18:1_**16:0**” as representing the transition 804.6 -> **255.2**, while “PC 16:0_**18:1**” will be understood as representing 804.6 -> **281.2**. It is essential to adhere to nomenclature for the application to apply the appropriate correction. For more examples, please refer to the highlighted lipid names in Figure 1, right panel.
- For sphingolipids long chain base-related transitions, the transitions are defined by the number **before** the separator. For example, Cer **d18:1**/16:0 will be understood as transition 538.5 -> **264.3**, while Cer **d16:1**/16:0 will be understood as transition 510.5 -> **236.4**.

Please refer to the provided templates to see more example of such nomenclature.

Table 1: Lipid classes, abbreviations, product ion types and MRM patterns used in LICAR.

Lipid class	Class abbreviations	Example name	Example MRM	Product ion type*	MRM pattern**
Lysophosphatidylcholine	LPC	LPC 20:3	546.4->184.1	Headgroup	LPC (Pos) Pro=184.1
Lysoplasmamanylcholine	LPC O-	LPC O-20:4	530.4->104.1	Headgroup	LPC-O (Pos) Pro=104.1
Lysoplasmamanylcholine	LPC O-	LPC O-20:4	530.4->184.1	Headgroup	LPC-O (Pos,qualifier) Pro=184.1
Lysophosphatidylethanolamine	LPE	LPE 18:1	480.3->339.3	Headgroup	LPE (Pos) Pre-Pro=141
Lysophosphatidylethanolamine	LPE	LPE 18:1	478.3->196.0	Headgroup	LPE (Neg) Pro=196
Phosphatidylcholine	PC	PC 32:0	734.6->184.1	Headgroup	PC (Pos) Pro=184.1
Phosphatidylcholine	PC	PC 16:0_18:1	804.6->281.2	FA	PC (Neg) FA
Phosphatidylethanolamine	PE	PE 38:4	768.6->627.5	Headgroup	PE (Pos) Pre-Pro=141
Phosphatidylethanolamine	PE	PE 38:4	766.6->196.0	Headgroup	PE (Neg) Pro=196
Phosphatidylethanolamine	PE	PE 18:0/20:4	766.6->303.2	FA	PE (Neg) FA
Plasmenylethanolamine	PE P-	PE P-18:1/20:4	750.5->361.3	FA	PE-P (Pos) FA
Phosphatidylinositol	PI	PI 36:2	880.6->603.6	Headgroup	PI (Pos) Pre-Pro=277
Phosphatidylinositol	PI	PI 36:2	880.6->241.0	Headgroup	PI (Neg) Pro=241
Phosphatidylinositol	PI	PI 18:1_18:1	861.5->281.2	FA	PI (Neg) FA
Phosphatidylserine	PS	PS 38:4	812.5->627.5	Headgroup	PS (Pos) Pre-Pro=185
Phosphatidylserine	PS	PS 38:4	810.5->723.5	Headgroup	PS (Neg) Pre-Pro=87
Phosphatidylserine	PS	PS 20:4_18:0	810.5->283.2	FA	PS (Neg) FA
Phosphatidylglycerol	PG	PG 36:2	792.6->603.5	Headgroup	PG (Pos) Pre-Pro=189
Phosphatidylglycerol	PG	PG 36:2	773.6->153.0	Headgroup	PG (Neg) Pro=153
Phosphatidylglycerol	PG	PG 18:1_18:1	773.5->281.2	FA	PG (Neg) FA
Sphingomyelin	SM	SM 34:1	703.6->184.1	Headgroup	SM (Pos) Pro=184.1
Ceramides	Cer	Cer d18:1/16:0	538.5->264.3	LCB	Cer (Pos) SphB-H2O
Dehydroxyceramides	dhCer	dhCer d18:0/22:0	624.6->284.3	LCB	dhCer (Pos) SphB
Monohexosylceramides	Hex1Cer	Hex1Cer d18:1/24:0	812.7->264.3	LCB	Hex1Cer (Pos) SphB-H2O
Dihexosylceramides	Hex2Cer	Hex2Cer d18:1/16:0	862.6->264.3	LCB	Hex2Cer (Pos) SphB-H2O

* See section 3.2

** See section 3.3

2.Start the application

2.1.Shinyapps.io

The easiest way the run LICAR is to use *via* Shinyapps.io.

LICAR is available at <https://slinghub.shinyapps.io/LICAR/>, where it can be used directly without any installation.

Alternatively, you may download and install the application by following steps 2.2. to 2.4 below.

2.2.Download the code

The R scripts and templates are provided as an RStudio project. You can download the Github repository (<https://github.com/SLINGhub/LICAR.git>) and open the Rstudio project. Alternatively, you can clone this repository using git, *e.g.* in RStudio.

2.3.Prerequisites

The following packages must be installed in R to run the scripts

- `enviPat`
- `stringr`
- `shiny`

2.4.Run the Shiny App

Open the script `LICAR_UI.R` in the R directory and click on the ‘Run App’ button.

3.Proceed with isotopic correction.

Upon starting the application, you will see its user interface, as shown in Figure 2.

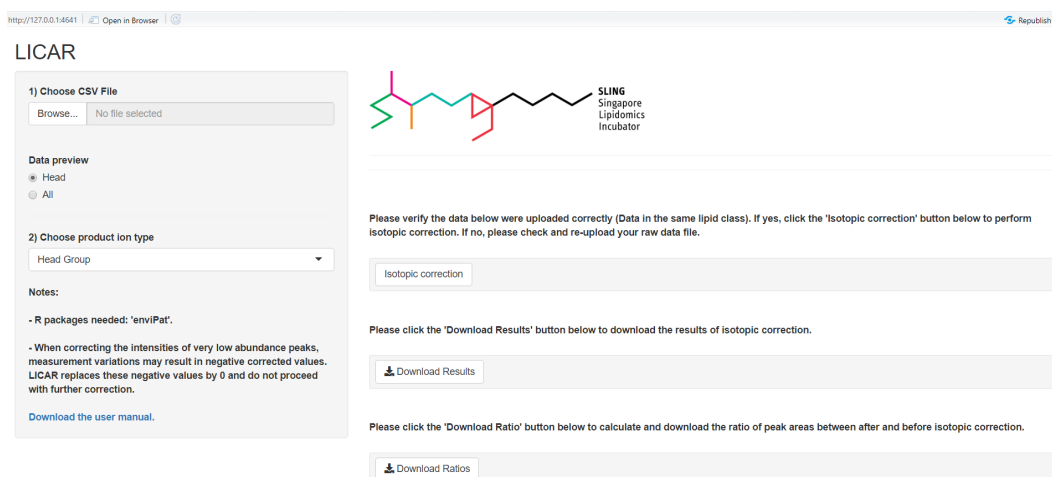


Figure 2: LICAR graphical user interface

3.1.Upload .csv file

Click the “Browse” button under “1) Choose CSV file” to upload your data. Navigate to the file of your choice and validate. Upon loading the .csv file, its content will be previewed in the application window, as shown in Figure 3. You may preview only the first few lines of the file (choose “Head” under “Data preview”) or its entirety (choose “All” under “Data preview”) in the right section of the user interface. You may thus verify that your data has been uploaded.

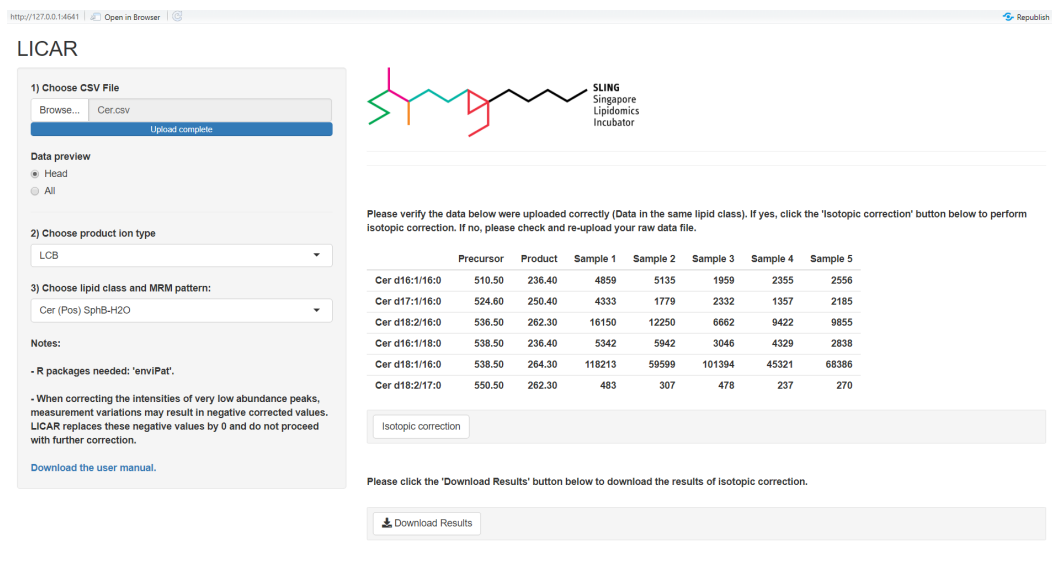


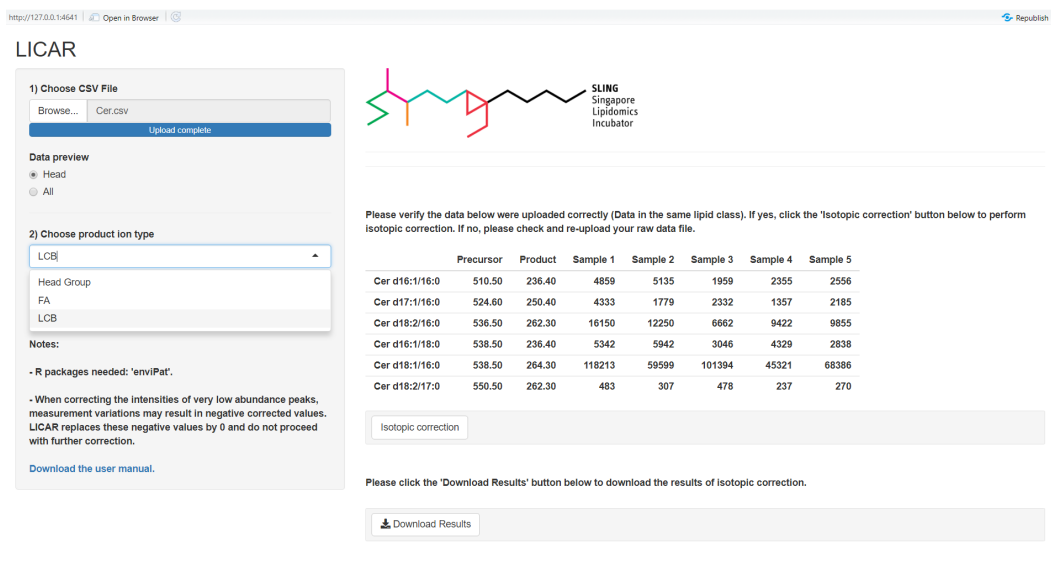
Figure 3: Data upload

3.2.Choose the type of product ion

Once your data is uploaded successfully, please choose the relevant type of product ion in the drop-down menu under “2) Choose product ion type”. There are three choices:

- **Head Group:** for headgroup related fragmentation (*e.g.* product ion of 184 for PC, neutral loss of 141 for PE...).
- **FA:** for fatty acyl-related product ions (*e.g.* fatty acid fragments of phospholipids in negative ionisation).
- **LCB:** for long chain base-related product ions (*e.g.* ceramides transitions as in Figures 3-5).

In the example in Figure 4, we choose “LCB” as we are correcting ceramides data.



LICAR

1) Choose CSV File

Browse... Cer.csv Upload complete

Data preview

Head

All

2) Choose product ion type

LCB

Head Group

FA

LCB

Notes:

- R packages needed: 'enviPat'.

- When correcting the intensities of very low abundance peaks, measurement variations may result in negative corrected values. LICAR replaces these negative values by 0 and do not proceed with further correction.

Download the user manual.

Please verify the data below were uploaded correctly (Data in the same lipid class). If yes, click the 'Isotopic correction' button below to perform isotopic correction. If no, please check and re-upload your raw data file.

	Precursor	Product	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Cer d16:1/16:0	510.50	236.40	4859	5135	1959	2355	2556
Cer d17:1/16:0	524.60	250.40	4333	1779	2332	1357	2185
Cer d18:2/16:0	536.50	262.30	16150	12250	6662	9422	9855
Cer d16:1/18:0	538.50	236.40	5342	5942	3046	4329	2838
Cer d18:1/16:0	538.50	264.30	118213	59599	101394	45321	68386
Cer d18:2/17:0	550.50	262.30	483	307	478	237	270

Isotopic correction

Please click the 'Download Results' button below to download the results of isotopic correction.

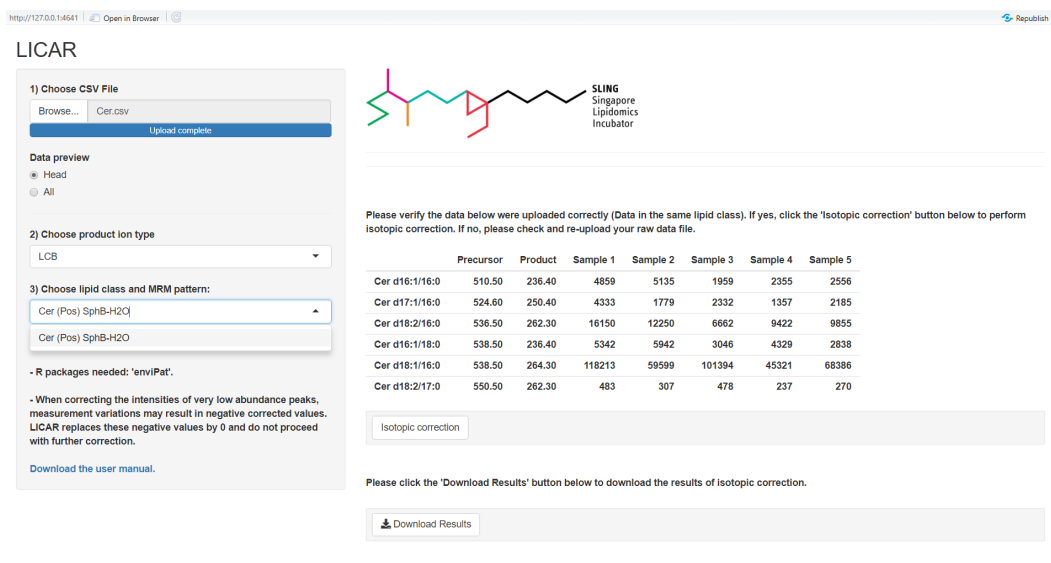
Download Results

Figure 4: Choosing product ion type

Choosing a wrong product ion type will result in the following error message: "Lipid class is wrong, please choose the class again!"

3.3. Specify the MRM pattern.

Upon selection of the product ion type, one last drop-down menu will appear asking the user to choose lipid class and MRM pattern. Although we have pre-set a total of 25 MRM transitions pattern for various lipid classes, the application pre-selects the relevant ones based on the uploaded data and the product ion type selected in section 3.2. In the example given in Figure 5, only one option remains. In other cases, two or three options may remain.



LICAR

1) Choose CSV File

Browse... Cer.csv Upload complete

Data preview

Head

All

2) Choose product ion type

LCB

3) Choose lipid class and MRM pattern:

Cer (Pos) SphB-H2O

Cer (Pos) SphB-H2O

Notes:

- R packages needed: 'enviPat'.

- When correcting the intensities of very low abundance peaks, measurement variations may result in negative corrected values. LICAR replaces these negative values by 0 and do not proceed with further correction.

Download the user manual.

Please verify the data below were uploaded correctly (Data in the same lipid class). If yes, click the 'Isotopic correction' button below to perform isotopic correction. If no, please check and re-upload your raw data file.

	Precursor	Product	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Cer d16:1/16:0	510.50	236.40	4859	5135	1959	2355	2556
Cer d17:1/16:0	524.60	250.40	4333	1779	2332	1357	2185
Cer d18:2/16:0	536.50	262.30	16150	12250	6662	9422	9855
Cer d16:1/18:0	538.50	236.40	5342	5942	3046	4329	2838
Cer d18:1/16:0	538.50	264.30	118213	59599	101394	45321	68386
Cer d18:2/17:0	550.50	262.30	483	307	478	237	270

Isotopic correction

Please click the 'Download Results' button below to download the results of isotopic correction.

Download Results

Figure 5: Choosing MRM pattern

We have pre-set 25 MRM transition patterns from various lipid classes commonly measured in our lab. However, advanced users can easily add their own MRM transition patterns for other lipid classes to extend the lipid coverage of our application.

3.4. Isotopic correction

After verifying the data and choosing the relevant fragmentation pattern and lipid class, click the “Isotopic correction” button to perform isotopic correction. The results of the correction appear in the application window, and get be exported as .csv files by clicking on “Download results”. (Figure 6)

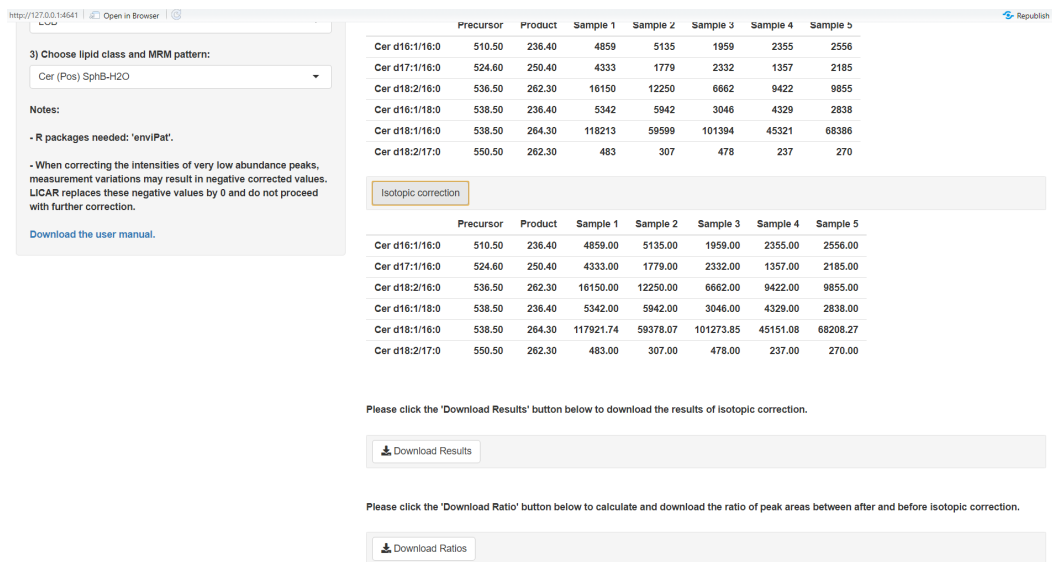


Figure 6: Isotopic correction results

The user can also export the result as ratio of peak areas between after and before isotopic correction, by clicking on “Download ratios”. These ratios, demonstrates the isotopic effect on specific lipid species, and can be a useful reference.