

## Supporting Information

### **“Strategies for Using Postcolumn Infusion of Standards to Correct for Matrix Effect in LC-MS-Based Quantitative Metabolomics”**

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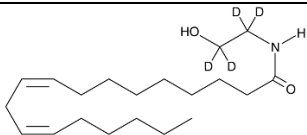
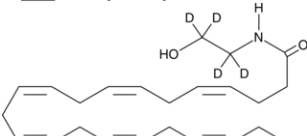
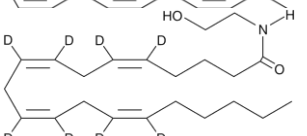
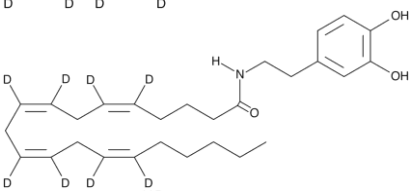
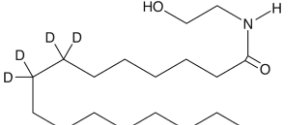
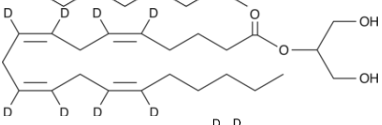
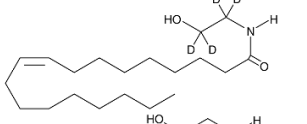
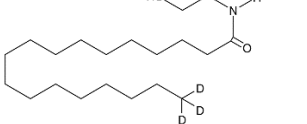
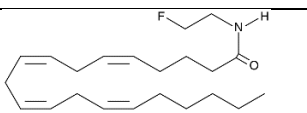
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**Supplementary Table S1.** Overview of structures and classifications of compounds used in this study.

| Analyte (labeled)                | Abbreviation (labeled) | Molecular structure (labeled)                                                       | Lipid maps ID <sup>1</sup> (unlabeled) | Sub class <sup>1</sup>                           |
|----------------------------------|------------------------|-------------------------------------------------------------------------------------|----------------------------------------|--------------------------------------------------|
| Linoleoyl Ethanolamide-d4        | d4-LEA                 |    | LMFA08040004                           | N-acyl ethanolamines (endocannabinoids) [FA0804] |
| Docosahexaenoyl Ethanolamide-d4  | d4-DHEA                |    | LMFA08040009                           | N-acyl ethanolamines (endocannabinoids) [FA0804] |
| Arachidonoyl Ethanolamide-d8     | d8-AEA                 |    | LMFA08040001                           | N-acyl ethanolamines (endocannabinoids) [FA0804] |
| N-Arachidonoyl Dopamine-d8       | d8-NADA                |    | LMFA08020084                           | N-acyl amines [FA0802]                           |
| Palmitoyl Ethanolamide-d4        | d4-PEA                 |   | LMFA08040013                           | N-acyl ethanolamines (endocannabinoids) [FA0804] |
| 2-Arachidonoyl Glycerol-d8       | d8-2AG                 |  | LMGL01010023                           | Monoacylglycerols [GL0101]                       |
| Oleoyl Ethanolamide-d4           | d4-OEA                 |  | LMFA08040015                           | N-acyl ethanolamines (endocannabinoids) [FA0804] |
| Stearoyl ethanolamide-d3         | d3-SEA                 |  | LMFA08040051                           | N-acyl ethanolamines (endocannabinoids) [FA0804] |
| PCI-IS                           | Abbreviation           | Molecular structure                                                                 | Lipid maps ID (unlabeled)              | Sub class (Lipid maps)                           |
| Arachidonoyl-2'-Fluoroethylamide | 2F-AEA                 |  | LMFA08020055                           | N-acyl amines [FA0802]                           |

Reference:

1. Fahy, E. *et al.* Update of the LIPID MAPS comprehensive classification system for lipids. *J. Lipid Res.* **50**, 9–14 (2009).

**Supplementary Table S2: LC-MS/MS conditions**

|                                       |  |                             |
|---------------------------------------|--|-----------------------------|
| <b>Autosampler temperature</b>        |  | 7 °C                        |
| <b>Oven temperature</b>               |  | 40 °C                       |
| <b>LC Flow rate</b>                   |  | 0.7 mL/min                  |
| <b>Divert valve switched to waste</b> |  | 0 – 0.8 min and 15 – 16 min |
| <b>Divert valve switched to MS</b>    |  | 0.8 – 15 min                |

| <b>Gradient</b> |                   |               |                  |
|-----------------|-------------------|---------------|------------------|
| <b>Time</b>     | <b>Module</b>     | <b>Events</b> | <b>Parameter</b> |
| 0.75            | Pumps             | Pump B Conc.  | 20%              |
| 0.95            | Pumps             | Pump B Conc.  | 26%              |
| 6               | Pumps             | Pump B Conc.  | 34%              |
| 7               | Pumps             | Pump C Conc.  | 1%               |
| 8               | Pumps             | Pump B Conc.  | 40%              |
| 10              | Pumps             | Pump B Conc.  | 54%              |
| 11              | Pumps             | Pump C Conc.  | 1%               |
| 12              | Pumps             | Pump B Conc.  | 56%              |
| 12              | Pumps             | Pump C Conc.  | 3%               |
| 13              | Pumps             | Pump B Conc.  | 78%              |
| 13              | Pumps             | Pump C Conc.  | 6%               |
| 14              | Pumps             | Pump B Conc.  | 85%              |
| 14              | Pumps             | Pump C Conc.  | 15%              |
| 14.5            | Pumps             | Pump B Conc.  | 85%              |
| 14.5            | Pumps             | Pump C Conc.  | 15%              |
| 14.8            | Pumps             | Pump B Conc.  | 20%              |
| 14.8            | Pumps             | Pump C Conc.  | 1%               |
| 16              | System controller | Stop          |                  |

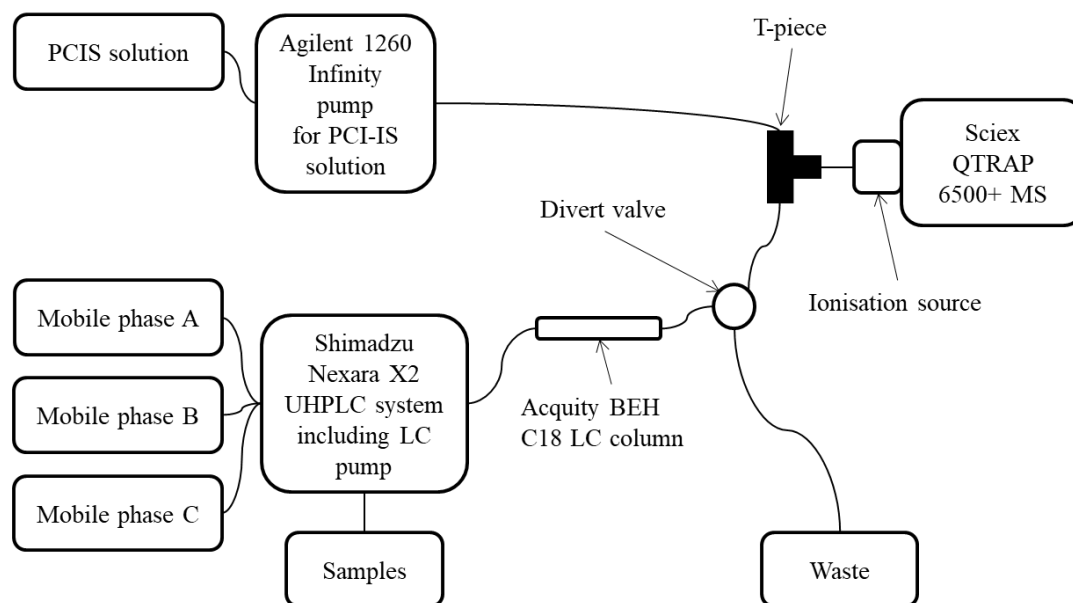
  

| <b>General MS parameters</b> |        |
|------------------------------|--------|
| <b>Experiment type</b>       | MRM    |
| <b>Curtain gas</b>           | 40     |
| <b>Gas 1</b>                 | 50     |
| <b>Gas 2</b>                 | 50     |
| <b>Ion Spray voltage</b>     | 4500   |
| <b>Temperature</b>           | 600 °C |

| <b>Compound specific MS parameters of the measured endocannabinoids and PCI-IS</b> |           |           |                        |                     |           |            |           |           |
|------------------------------------------------------------------------------------|-----------|-----------|------------------------|---------------------|-----------|------------|-----------|-----------|
| <b>Name</b>                                                                        | <b>Q1</b> | <b>Q3</b> | <b>Rt center (min)</b> | <b>Rt width (s)</b> | <b>CE</b> | <b>CXP</b> | <b>DP</b> | <b>EP</b> |
| d4-LEA                                                                             | 328.300   | 66.200    | 12.9                   | 30                  | 36        | 11         | 74        | 10        |
| d4_DHEA                                                                            | 376.300   | 66.200    | 12.9                   | 30                  | 25        | 11         | 70        | 10        |
| LEA                                                                                | 324.000   | 62.000    | 12.9                   | 30                  | 36        | 11         | 74        | 10        |
| AEA                                                                                | 348.000   | 62.000    | 13                     | 30                  | 38        | 11         | 72        | 10        |
| d8-AEA                                                                             | 356.300   | 62.200    | 12.9                   | 30                  | 42        | 11         | 70        | 10        |
| DHEA                                                                               | 372.000   | 62.000    | 12.9                   | 30                  | 25        | 11         | 70        | 10        |
| d8-2-AG                                                                            | 387.300   | 294.200   | 13.2                   | 30                  | 20        | 11         | 56        | 10        |
| 1/2-AG                                                                             | 379.210   | 287.000   | 13.2                   | 30                  | 19        | 11         | 56        | 10        |
| d4-PEA                                                                             | 304.300   | 62.200    | 13.2                   | 30                  | 36        | 11         | 78        | 10        |
| PEA                                                                                | 300.000   | 62.000    | 13.2                   | 30                  | 36        | 11         | 78        | 10        |
| d3-SEA                                                                             | 331.300   | 62.200    | 13.7                   | 30                  | 25        | 11         | 72        | 10        |
| SEA                                                                                | 328.000   | 62.000    | 13.7                   | 30                  | 31        | 11         | 72        | 10        |
| NADA                                                                               | 440.000   | 137.000   | 13.3                   | 30                  | 34        | 11         | 75        | 10        |
| d8-NADA                                                                            | 448.400   | 137.000   | 13.3                   | 30                  | 34        | 11         | 75        | 10        |
| OEA                                                                                | 326.000   | 62.000    | 13.4                   | 30                  | 30        | 11         | 72        | 10        |
| d4-OEA                                                                             | 330.300   | 66.200    | 13.4                   | 30                  | 30        | 11         | 72        | 10        |
| 2F-AEA                                                                             | 350.300   | 269.200   | 7.5                    | 900                 | 20        | 11         | 50        | 10        |

**Supplementary Figure S1:** Schematic drawing of the LC-MS/MS instrument setup with PCIS



## Supplementary Figure S2: PCIS correction outperforming SIL-ISTD correction due to retention time-shift.

Examples of retention time difference between a deuterated standard and its non-labeled analogue in a region of variable ion suppression, shown by overlaid MRM-transitions of the PCIS (grey), a deuterated standard (blue) and its non-labeled analogue (orange) in a plasma calibration standard. A: d4-LEA elutes earlier than LEA and is more affected by ion suppression (around 12.9 min). The d4-LEA peak is therefore smaller than it would be if the ion suppression was the same as for LEA, resulting in an overcorrection when using d4-LEA to correct for matrix effect by peak area correction. B: d8-2AG elutes earlier than 2-AG and suffers less from ion suppression (around 13.3 min). The d8-2-AG peak is therefore larger than it would be if the ion suppression was the same as for 2-AG, resulting in an undercorrection when using d8-2-AG to correct for matrix effect by peak area correction.

