A Simple and Highly Sensitive Quantitation of Eicosanoids in Biological Samples Using Nano-flow Liquid Chromatography/Mass Spectrometry

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A simple sample preparation method for eicosanoids was developed by the combination of deproteinization and nanoLC-ESI-MS/MS. Eicosanoids are a group of bioactive lipid mediators, present in trace amounts in the body. Therefore, an analytical method for eicosanoids requires superior sensitivity. The method described in this report, which takes advantage of the highly sensitive power of nanoLC-ESI-MS/MS, enabled a simplification of the sample-preparation process. Eicosanoid extraction was performed just by homogenization in methanol with subsequent phospholipid removal, and then the liquid phase was directly subjected to nanoLC-ESI-MS/MS analysis without a condensation process. The quantitation range achieved 0.01 – 100 ng/mL for thromboxane B2, and 0.05 – 100 ng/mL for prostaglandin E2, prostaglandin D2, prostaglandin F2, leukotriene B4, 6-keto prostaglandin F1α, and 11-dehydro thromboxane B2. Rat brain sample analyses demonstrated the feasibility of the quantification of those seven eicosanoids from biological samples.

Keywords Sample preparation, eicosanoids, nanoLC-ESI-MS/MS

(Received August 7, 2017; Accepted September 22, 2017; Published February 10, 2018)
preparation for eicosanoid analysis by utilizing nanoLC-ESI-MS/MS. NanoLC-ESI-MS/MS has been established, especially in the proteomics field, as the standard methodology for the quantitation and identification of small amounts of proteins because of its superior sensitivity compared to conventional LC/MS/MS. Recently, the technology has been expanded to small molecule analyses and metabolomics research, mostly for relatively abundant metabolites. The highly sensitive power of nanoLC-ESI-MS/MS should also be beneficial for trace component analysis as well. We hypothesized that the sensitivity of nanoLC-ESI-MS/MS could compensate for the minimized molecule analyses and metabolomics research, mostly for relatively abundant metabolites. The highly sensitive power of nanoLC-ESI-MS/MS should also be beneficial for trace component analysis as well. We hypothesized that the sensitivity of nanoLC-ESI-MS/MS could compensate for the minimized sample preparation. This approach was validated to be an easy and quantitative method using rat brain samples.

**Experimental**

**Chemicals, reagents, and materials**

Acetonitrile, isopropanol, methanol, acetone, acetic acid, and formic acid (all LC/MS grade) were purchased from Wako Pure Chemical (Osaka, Japan). Prostaglandin E₂ (PGE₂), prostaglandin D₂ (PGD₂), 6-keto prostaglandin F₁α (6-keto PGF₁α), prostaglandin F₂α (PGF₂α), leukotriene B₄ (LTB₄), thromboxane B₂ (TXB₂), and 11-dehydro thromboxane B₂ (11-dehydro TXB₂) were purchased from Cayman Chemical Co., Ltd. (Ann Arbor, MI, USA). These reagents were dissolved in methanol, and standard mixture solutions were prepared at 0.1, 0.5, 1, 5, 10, 50, 100, 500, and 1000 ng/mL. PGE₂-d₅, PGD₂-d₅, LTB₄-d₅, 6-keto PGF₁α-d₅, PGF₂α-d₅, TXB₂-d₅, and 11-dehydro TXB₂-d₅ were also purchased from Cayman Chemical Co., Ltd. These deuterium-labeled reagents were dissolved in methanol (10 ng/mL each) and used as internal standards (IS) solutions. Rat brain and plasma (Crl:CD, 8-week-old males) were purchased from Japan SLC, Inc. (Shizuoka, Japan). Charcoal stripped human EDTA plasma was purchased from BioChemed services (Winchester, VA). Fatty acid-free human serum albumin was purchased from Sigma-Aldrich (St. Louis, MO) and diluted with Milli-Q water (4%, w/v). All of these standard solutions and samples were frozen at –80°C until use.

**Nano-flow LC/MS/MS condition**

A Dionex Ultimate 3000 nano-flow liquid chromatography system (Thermo Fisher Scientific Inc., San Jose, CA, USA) which consists of a high-pressure binary pump, a column oven, and a 6-port switching valve was used for chromatographic separation. Monolithic columns were selected as trap (Monocap for fast flow, 100 mm × 50 μm i.d., Kyoto Monotech, Kyoto, Japan) and separation columns (Monocap for nano flow, 300 mm × 50 μm i.d., Kyoto Monotech). The column temperature was maintained at 50°C. Samples were loaded onto the trap column using a loading pump with acetic acid/Milli-Q water (0.1/100, v/v) at a flow rate of 5 μL/min. After 10 min of trapping, the 6-port valve was switched to the separation mode, and a gradient elution was performed with 0.1% acetic acid in Milli-Q water (solvent A) and 0.1% acetic acid in acetonitrile: isopropanol:acetone (90:5:5, solvent B) at a flow rate of 200 nL/min. The gradient was used as follows (time, %B): 0 min, 2%; 10 min, 2%; 30 min, 5%; 50 min, 50%; 60 min, 98%; 75 min, 98%; 75.1 min, 2%; 85 min, 2%. The eluate from the separation column was directly introduced to a nano-electrospray needle (FS360-50-15-D, New Objective Inc., MA, USA) and ionized in negative ionization mode. The ion source conditions were set as follows: the spray voltage was 1750 V, the heated-capillary temperature was 270°C, and the sheath gas (N₂) pressure was 30 psi. A TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific) was used for eicosanoid detection in a selected reaction monitoring mode (SRM). Eicosanoids mostly produce [M–H]⁻ ion by negative ionization; thus, the [M–H]⁻ ion was set as Q1. Q3 was selected based on the intensity and the specificity of the target molecules; however, there are some eicosanoids whose Q1/Q3 are the same because of their structural similarities; therefore, chromatographic separation is necessary to distinguish isomeric molecules, such as PGE₂ and PGD₂. Table 1 contains a list of the mass-spectrometry parameters for seven eicosanoids and deuterium-labeled internal standards whose chromatograms were acquired by the timed-SRM mode with a 2-min scheduled time window and a 1.5-s cycle time. Mass resolutions for Q1 and Q3 were set at 0.7 Da full-width at the half-maximum (FWHM) for all molecules. The Xcalibur software package (Thermo Fisher Scientific) was used for peak detection, integration, and quantitative calculations of the mass spectrometry data.

**Sample preparation**

Frozen brain tissue was weighed and transferred to a test tube in ice. To exclude any differences between individuals and brain regions, the experiments were carried out using one pooled

| Name                | Q1 (m/z) | Q3 (m/z) | Collision energy/V | S-lens/V | RT/min | RSD, % | Peak area |
|---------------------|----------|----------|--------------------|----------|--------|--------|-----------|
| PGE₂                | 351.2    | 271.1    | 18                 | 83       | 43.0   | 0.11   | —         |
| PGD₂                | 351.2    | 271.1    | 19                 | 83       | 42.2   | 0.12   | —         |
| PGF₂α               | 353.2    | 193.2    | 22                 | 133      | 41.2   | 0.05   | —         |
| 6-keto-PGF₁α        | 369.2    | 163.2    | 27                 | 109      | 36.3   | 0.08   | —         |
| TXB₂                | 369.1    | 169.1    | 18                 | 85       | 39.9   | 0.09   | —         |
| 11-Dehydro-TXB₂     | 367.1    | 161.1    | 18                 | 112      | 42.5   | 0.06   | —         |
| LTB₄                | 335.1    | 195.1    | 18                 | 80       | 51.1   | 0.06   | —         |
| PGE₂-d₅             | 355.2    | 275.2    | 18                 | 83       | 43.0   | 0.12   | 9.7       |
| PGD₂-d₅             | 355.2    | 275.2    | 19                 | 83       | 42.1   | 0.14   | 12.2      |
| PGF₂α-d₅            | 357.2    | 197.2    | 22                 | 133      | 41.2   | 0.05   | 5.7       |
| 6-keto-PGF₁α-d₅     | 373.2    | 167.1    | 27                 | 109      | 36.3   | 0.08   | 8.3       |
| TXB₂-d₅             | 373.1    | 173.1    | 18                 | 85       | 39.9   | 0.09   | 6.9       |
| 11-Dehydro-TXB₂-d₅  | 371.1    | 165.1    | 18                 | 112      | 42.5   | 0.10   | 6.7       |
| LTB₄-d₅             | 339.1    | 197.1    | 18                 | 80       | 51.0   | 0.05   | 8.0       |
homogenate. The tissue sample was homogenized in three volumes (v/w) of ice-cold formic acid:methanol (0.5:99.5) by a ball mill (Retsch GmbH, Haan, Germany). A 400-μL sample was mixed with 10 μL of an IS solution, and then transferred to a Captiva ND Lipids 96-well plate followed by centrifugation at 2000g for 2 min. Finally, the filtrated sample was directly injected to the nanoLC-ESI-MS/MS for analysis. For recovery and spike tests, a certain amount of standard solution (0, 25, and 250 ng/mL) was spiked into pooled brain homogenate to make the added concentration to be 0, 2.5, and 25 ng/g tissue.

**Extraction efficiency test**

The extraction efficiency was evaluated with a deuterium-labeled standard spiked into plasma. A 50-μL aliquot of rat plasma was transferred to a test tube (2.0 mL) in ice and mixed with 5 μL of IS solution to achieve a final concentration of 10 ng/mL. The sample was homogenized with 150 μL of ice-cold formic acid:methanol (0.5:99.5) or 150 μL of ice-cold formic acid:acetonitrile (0.5:99.5). The sample was transferred to a Captiva ND Lipids 96-well plate (Agilent Technologies, CA, USA) for efficient removal of the proteins and phospholipids, followed by centrifugation at 2000g for 2 min. Then, 10 μL of flow trough was directly applied for LC/MS/MS analysis.

**Quantitation method**

Calibration standards were prepared by diluting the standard stock solutions in methanol to final concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, and 100 ng/mL for all molecules. Fifty microliters of methanol were transferred to a test tube (2.0 mL) in ice and mixed with 5 μL of IS solution, 5 μL of methanol, and 150 μL of ice-cold formic acid:methanol (0.5:99.5). The samples were vortex mixed for 1 min, and then all of the homogenate solution was transferred to a Captiva ND Lipids 96-well plate, followed by centrifugation at 2000g for 2 min. Finally, 10 μL of the flow trough was directly applied for LC/MS/MS analysis. The calibration curve was calculated by a least-square linear regression method with 1/x weighting. The quantitative accuracy and precision was evaluated by a standard solution prepared at three different concentrations (final concentration: 0.2, 2 and 20 ng/mL, n = 3).

**Recovery rates**

Recovery rates were determined by comparing the peak areas of deuterium-labeled standards that were added to the brain both before (pre-spiked) and after (post-spiked) sample preparation. Pre-spiked samples were prepared as follows: deuterium-labeled standards were spiked in 50 μL of brain homogenate at 5 ng/mL, and then followed by the sample-preparation procedure described above. After extraction, 5 μL of methanol was added to the eluate. For post-spiked samples, deuterium labeled standards were added after sample preparation. The recovery rate was calculated as follows: recovery rate (%) = (peak area of pre-spiked standard)/(peak area of post-spiked standard) × 100.

**Results and Discussion**

**Chromatographic separation**

Eicosanoid is a group of lipid mediator synthesized from arachidonic acid, called an arachidonic acid cascade, mainly consisting of 6 different bioactive molecules: PGE\textsubscript{2}, PGD\textsubscript{2}, PGF\textsubscript{2α}, PGH\textsubscript{2}, TXA\textsubscript{2}, and LTA\textsubscript{4}. Some of them are quickly metabolized to stable forms in a body, such as 6-keto-PGF\textsubscript{1α} from PGH\textsubscript{2}, TXB\textsubscript{2} and 11-dehydroxy-TXB\textsubscript{2} from TXA\textsubscript{2}, and LTB\textsubscript{4} from LTA\textsubscript{4}, which can be indicators of each pathway.

Therefore, seven eicosanoids (PGE\textsubscript{2}, PGD\textsubscript{2}, PGF\textsubscript{2α}, 6-keto-PGF\textsubscript{1α}, TXB\textsubscript{2}, 11-dehydroxy-TXB\textsubscript{2} and LTB\textsubscript{4}) were selected as target molecules for analytical evaluation in this study as indicators of inflammatory responses derived from arachidonic acid cascade.

The mass-spectrometry parameters of all the target analytes are listed in Table 1. PGE\textsubscript{2} and PGD\textsubscript{2} had the same Q1 and Q3 value, and the other parameters were also almost identical; therefore, mass spectrometry alone could not differentiate these two molecules. Figure 1 shows a nanoLC-ESI-MS/MS chromatogram of a standard mixture containing seven eicosanoids (25 pg on column). A monolith chromatographic column was selected for the nano-LC separation with an 85-min runtime, including a 10-min preconcentration step. The chromatographic conditions enabled the separation of PGE\textsubscript{2} and PGD\textsubscript{2} at 42.2 and 43.0 min respectively. A shoulder peak was observed in TXB\textsubscript{2} chromatogram. A similar chromatogram was reported in other articles.\textsuperscript{26,31} We currently speculated that these peaks were derived from multi form TXB\textsubscript{2}; with the equilibrium state. The relative standard deviations (RSD) of the retention times of the analytes were all less than 0.2% (Table 1) and the RSD of the peak area of the deuterium labeled standards were all less than 13.0%, indicating the robustness of the chromatographic conditions (n = 16).

**Sample preparation**

Methanol or acetonitrile was generally used for a water-soluble organic solvent based protein precipitation; however, the extraction efficiency could be different depending on the molecule of interest. A comparison of the LC/MS/MS peak area between methanol extraction and acetonitrile extraction for stable isotope-labeled PGE\textsubscript{2}, PGD\textsubscript{2}, and LTB\textsubscript{4} spiked in rat plasma was conducted. PGE\textsubscript{2} and PGD\textsubscript{2} revealed no significant differences, whereas methanol exhibited a larger intensity for LTB\textsubscript{4} extraction (Fig. 2, sample preparation method is given in
section Extraction efficiency test). This difference could be the result of both the extraction efficiency and the matrix effect derived from interferences in the extract. The lower extraction efficiency of LTB4 in acetonitrile could be mainly derived from a combination of these two factors. The major cause was not identified in this study; however, based on comparison data, methanol was selected as a suitable solvent for sample preparation.

**Recovery rates**

The recovery rates in the sample preparation process and the analytical reproducibility of all target molecules were determined using stable isotope-labeled standards spiked into rat plasma. The recovery rates of all molecules were greater than 70% (Table 2). The analytical reproducibility, evaluated by the relative standard deviation (RSD) of seven replicates, were all less than 15%. In a previous report, the recovery rates of eicosanoid by LLE were 10.61% for TXB2, 25.56% for 11-dehydro TXB2, 35.19% for PGF2α, 33.21% for PGD2, 49.19% for PGE2, and 12.75% for 6-keto PGF1α extracted with two-step LLE (the first extraction was chloroform/isopropanol (2:1); the second extraction was with methyl tert-butyl ether)32 and 22.1% for PGD2, 26.5% for PGE2, 21.1% for LTB4, and 29.5% for PGF2α when extracted with 1 M acetic acid/2-isopropanol/hexane (2:20:30, v/v/v).21 As for the recovery rate of eicosanoid using a SPE column, more than 65% by weak anion-exchange, 0 - 54% by strong anion-exchange and more than 68% by octadecylsilyl based column was reported.12 On the other hand, the recovery rate of our result was shown to be equal or above that of the SPE and LLE method. Taken together, nanoLC-ESI-MS/MS detection coupled with methanol extraction, and phospholipid removal was demonstrated to be a robust, reliable method for quantitative analysis.

### Quantitative validation

The quantification of the seven eicosanoids was carried out using calibration curves prepared from standard solutions and stable isotope labeled internal standards using methanol as the blank matrix. A suitable blank matrix for endogenous molecule quantitation is the same or equivalent biological matrix without the target analytes. We first tested two protein solutions, fatty acid free human serum albumin solution (4%) and charcoal stripped human plasma, but LTB4 was detected in both (Fig. 3). Thus, methanol was selected as a blank matrix for the calibration curve because methanol was also used in the sample preparation.

A linear dynamic range from 0.01 to 100 ng/mL (50 fg to 500 pg on column) was achieved for TXB2, and from 0.05 to 100 ng/mL (250 fg to 500 pg on column) for PGE2, PGD2, LTB4, 6-keto PGF1α, 11-dehydroTXB2, and PGF2α and correlation coefficients of r >0.99 were obtained for all analytes (Table 3). The sensitivity was almost equivalent to the detection limits reported in other articles: 0.3 ± 0.1 pg on column for PGE2 and PGD2,28 10 pg on column for PGD2, 5 pg on column for PGF2α and TXB2, 50 pg on column for 6-keto PGF1α,33 and 4 pg on column for LTB4.34 To evaluate the validity of the calibration curve, spike-and-recovery tests were performed. For this purpose, rat brain was selected as a typical difficult-to-treat matrix because of the richness in lipids and proteins, which makes sample preparation difficult for removing those abundant constituents. Therefore, brain was considered to be an appropriate matrix to demonstrate the feasibility of the new procedure. Rat brain was mixed with specific amounts of standards (0, 2.5 and 25 ng/g tissue), and applied to the quantitative analysis. As a result, all eicosanoids were observed from the rat brain within the calibration range. The quantitative robustness of this method was exemplified based on the

### Table 2 Extraction recovery of eicosanoids from rat plasma (n = 3)

| Analyte              | Recovery, % | RSD, % |
|----------------------|-------------|--------|
| 6-keto-PGF1α-d₄      | 84.8        | 13.4   |
| PGE₂-d₄              | 75.0        | 3.4    |
| Thromboxane B₂-d₄    | 70.2        | 2.5    |
| PGF2α-d₄             | 73.3        | 2.7    |
| PGD₂-d₄              | 77.2        | 1.5    |
| 11-Dehydro-TXB₂-d₄   | 72.7        | 0.8    |
| Leukotriene B₄-d₄    | 74.8        | 2.5    |

Fig. 2 Comparison between methanol extraction (gray bar) and acetonitrile extraction (black bar) (n = 3).

Fig. 3 LTB4 chromatogram of a) deuterium labeled LTB4, b) extract of charcoal stripped human serum albumin, c) extract of fatty acid free albumin, and d) methanol. Each black arrow in the figure indicates the peak of LTB₄.
accuracies of spiked concentrations of all eicosanoids, 82.2 – 117.6% and RSD values less than 15% (Table 4). Moreover, the endogenous concentration observed in this experiment was almost equivalent to the values in other reports.35 Taken together, this study demonstrated the feasibility of this method for sample preparation and quantitation.

Conclusions

Many previous reports stated that the precise and accurate analysis of eicosanoids in vitro and in vivo is challenging due to their low concentration and the existence of structural isomers, making specific detection difficult. In addition, the analytical procedures in those reports required laborious sample-preparation processes. In this study, a combination of protein precipitation, phospholipid removal, and nanoLC-ESI-MS/MS was confirmed to be an improved approach, especially in sample preparation. The minimized sample-preparation process reduced the sample preparation time and contributed to the consistent and reproducible results. The accuracy and precision observed in the analysis of rat-brain samples exemplified the data quality. In this study, nanoLC-ESI-MS/MS required a long run time of 85 min for the chromatographic separation of PGD2 and PGE2. For practical situations, a shorter run time analysis is demanded; however, this study demonstrated that the enrichment process by SPE or LLE is not necessary, even for eicosanoid analysis. In summary, we demonstrated that a robust quantification of endogenous metabolites could be carried out by nanoLC-ESI-MS/MS with simple sample preparation, indicating that this approach can be expanded to other metabolites that require highly sensitive analysis.

Table 3 Calibration range of seven target analytes determined by the calibration curve

| Nominal concentration (ng/mL matrix) | TXB2 | PGE2 | PGD2 | LTB4 | 6-keto-PGF1α | 11-Dehydro TXB2 | PGF2α |
|-------------------------------------|------|------|------|------|--------------|-----------------|--------|
|                                     | Accuracy, % | ng/mL | Accuracy, % | ng/mL | Accuracy, % | ng/mL | Accuracy, % | ng/mL | Accuracy, % | ng/mL | Accuracy, % | ng/mL | Accuracy, % | ng/mL |
| 0.01                                | 114   | 0.01 | ——     | ——   | ——          | ——   | ——            | ——   | ——        | ——   |
| 0.05                                | 106   | 0.05 | 83     | 0.04 | 111         | 0.06 | 99            | 0.05 | 92        | 0.05 | 100         | 0.05 | 106         | 0.05 |
| 0.1                                 | 97    | 0.10 | 110    | 0.11 | 84          | 0.08 | 83            | 0.08 | 99        | 0.10 | 91          | 0.09 | 119         | 0.12 |
| 0.5                                 | 95    | 0.47 | 106    | 0.53 | 103         | 0.51 | 95            | 0.47 | 102       | 0.51 | 98          | 0.49 | 100         | 0.50 |
| 1                                  | 92    | 0.92 | 102    | 1.02 | 100         | 1.00 | 101           | 1.01 | 102       | 1.02 | 102         | 1.02 | 110         | 1.10 |
| 5                                  | 91    | 4.54 | 102    | 5.09 | 113         | 5.67 | 106           | 5.32 | 103       | 5.14 | 104         | 5.18 | 88          | 4.42 |
| 10                                 | 101   | 10.13| 98     | 9.79 | 112         | 11.17| 111           | 11.08| 103       | 10.29| 102         | 10.18| 101         | 10.13|
| 50                                 | 104   | 52.20| 99     | 49.34| 103         | 51.57| 100           | 50.11| 99        | 49.67| 100         | 50.07| 107         | 53.26|
| 100                                | 98    | 98.23| 101    | 100.73| 97         | 96.66| 99            | 98.52| 100       | 99.87| 99          | 99.57| 97          | 97.08|

Table 4 Accuracy and precision of the spike-and-recovery tests of whole rat brains (0, 2.5, and 25 ng/g tissue, n = 4)

| Nominal concentration (ng/g of brain tissue) | #01 | #02 | #03 | #04 | Mean (ng/g of brain tissue) | Accuracy, % | RSD, % |
|---------------------------------------------|-----|-----|-----|-----|-----------------------------|-------------|--------|
| PGE2                                         |     |     |     |     |                             |             |        |
| 0                                           | 4.40| 3.57| 4.15| 3.70| 3.95                        | 9.8         |        |
| 2.5                                         | 7.00| 6.67| 5.99| 6.67| 6.58                        | 105.1       | 6.5    |
| PGD2                                         |     |     |     |     |                             |             |        |
| 0                                           | 34.80|37.31| 35.22|37.55|36.22                        | 3.9         |        |
| 2.5                                         | 39.14|35.83| 40.30|40.39|38.92                        | 107.8       | 5.5    |
| PGF2α                                       |     |     |     |     |                             |             |        |
| 0                                           | 63.30|57.95| 63.83|61.11|61.55                        | 101.3       | 4.3    |
| 2.5                                         | 7.77| 7.90| 7.70| 7.66| 7.75                        | 1.3         |        |
| 6-keto-PGF1α                                 |     |     |     |     |                             |             |        |
| 0                                           | 37.86|27.50| 32.91|38.21|34.12                        | 105.5       | 14.8   |
| 2.5                                         | 9.17| 8.84| 8.85| 9.35| 9.05                        | 2.8         |        |
| TXB2                                         |     |     |     |     |                             |             |        |
| 0                                           | 34.11|31.14| 34.55|37.70|34.37                        | 101.3       | 7.8    |
| 2.5                                         | 15.70|16.82| 16.25|15.59|16.09                        | 3.5         |        |
| 11-Dehydro-TXB2                             |     |     |     |     |                             |             |        |
| 0                                           | 41.72|44.87| 43.84|43.82|43.56                        | 109.9       | 3.0    |
| 2.5                                         | 2.90| 3.47| 2.60| 2.99| 2.99                        | 117.5       | 12.0   |
| LTB4                                         |     |     |     |     |                             |             |        |
| 0                                           | 2.96| 2.99| 3.20| 2.94| 3.02                        | 117.6       | 4.1    |
| 25                                          | 23.47|27.18| 26.13|27.15|25.99                        | 103.6       | 6.7    |
References

1. P. T. Bozza, I. Bakker-Abreu, R. A. Navarro-Xavier, and C. Bandeira-Melo, *Prostaglandins Leukot. Essent. Fat. Acids, 2011*, 85, 205.
2. P. C. Calder, *Biochimie, 2009*, 91, 791.
3. E. A. Dennis and P. C. Norris, *Nat. Rev. Immunol., 2015*, 15, 511.
4. K. Wanggren, A. Stavreus-Evers, C. Olsson, E. Andersson, and K. Gernzell-Danielsson, *Hum. Reprod., 2008*, 23, 2359.
5. C. E. Trebino, J. L. Stock, C. P. Gibbons, B. M. Naiman, T. S. Wachtman, J. P. Umland, K. Pandher, J.-M. Lapointe, S. Saha, M. L. Roach, D. Carter, N. A. Thomas, B. A. Durtzchi, J. D. McNeish, J. E. Hambor, P.-J. Jakobsson, T. J. Carty, J. R. Perez, and L. P. Audoly, *Proc. Natl. Acad. Sci., 2003*, 100, 9044.
6. S. Narumiya, F. Ushikubi, E. Segi, Y. Sugimoto, T. Murata, T. Matsuoka, T. Kobayashi, H. Hizaki, K. Tuboi, M. Katsuyama, A. Ichikawa, T. Tanaka, and N. Yoshida, *Nature, 1998*, 395, 281.
7. M. Divangahi, D. Desjardins, C. Nunes-Alves, H. G. Remold, and S. M. Behar, *Nat. Immunol., 2010*, 11, 751.
8. M. Balazy and R. C. Murphy, *Anal. Chem., 1986*, 58, 1098.
9. W. Liu, J. D. Morrow, and H. Yin, *Free Radic. Biol. Med., 2009*, 47, 1101.
10. F. Shono, K. Yokota, K. Horie, S. Yamamoto, K. Yamashita, K. Watanabe, and H. Miyazaki, *Anal. Biochem., 1988*, 168, 284.
11. M. Reinke, *Am. J. Physiol., 1992*, 262, E658.
12. A. I. Ostermann, I. Willenberg, and N. H. Schebb, *Anal. Bioanal. Chem., 2015*, 407, 1403.
13. L. Kortz, J. Dorow, S. Becker, J. Thiery, and U. Ceglarek, *J. Chromatogr. B, 2013*, 927, 209.
14. A. Lubin, S. Geerincx, S. Bajic, D. Cabooter, P. Augustijns, F. Cuyckens, and R. J. Vreeken, *J. Chromatogr. A, 2016*, 1440, 260.
15. M. S. Gachet, P. Rhyn, O. G. Bosch, B. B. Quednow, and J. Gertsch, *J. Chromatogr. B, 2015*, 976-977, 6.
16. N. Wang, R. Dai, W. Wang, Y. Peng, X. Zhao, and K. Bi, *Talanta, 2016*, 161, 157.
17. A. Sasaki, H. Fukuda, N. Shiida, N. Tanaka, A. Furugen, J. Ogura, S. Shuto, N. Mano, and H. Yamaguchi, *Anal. Bioanal. Chem., 2015*, 407, 1625.
18. M. Takabatake, T. Hishinuma, N. Suzuki, S. Chiba, H. Tsukamoto, H. Nakamura, T. Saga, Y. Tomioka, A. Kurose, T. Sawai, and M. Mizugaki, *Prostaglandins Leukot. Essent. Fatty Acids, 2002*, 67, 51.
19. Y. Wang, A. M. Armando, O. Quenenberger, C. Yan, and E. A. Dennis, *J. Chromatogr. A, 2014*, 1359, 60.
20. E. C. Soo, A. J. Aubry, S. M. Logan, P. Guerry, J. F. Kelly, N. M. Young, and P. Thibault, *Anal. Chem., 2004*, 76, 619.
21. S. R. Dahl, C. R. Kleiveland, M. Kassem, T. Lea, E. Lundanes, and T. Greibrokk, *J. Sep. Sci., 2008*, 31, 2627.
22. Z. D. Peterson, D. C. Collins, C. R. Bowerbank, M. L. Lee, and S. W. Graves, *J. Chromatogr. B, 2002*, 776, 221.
23. B. Rago and C. Fu, *J. Chromatogr. B, 2013*, 936, 25.
24. Y. Kita, T. Takahashi, N. Uozumi, and T. Shimizu, *Anal. Biochem., 2005*, 342, 134.
25. J. Peng, J. E. Elias, C. C. Thoreen, L. J. Licklider, and S. P. Gygi, *J. Proteome Res., 2003*, 2, 43.
26. Y. Ishihama, J. Rappold, I. S. Andersen, and M. Mann, *J. Chromatogr. A, 2002*, 797, 233.
27. L. J. Licklider, C. C. Thoreen, J. Peng, and S. P. Gygi, *Anal. Chem., 2002*, 74, 3076.
28. K. T. Myint, K. Aoshima, S. Tanaka, T. Nakamura, and Y. Oda, *Anal. Chem., 2009*, 81, 1121.
29. T. M. Khin, T. Uehara, K. Aoshima, and Y. Oda, *Anal. Chem., 2009*, 81, 7766.
30. T. Uehara, A. Yokoi, K. Aoshima, S. Tanaka, T. Kadowaki, M. Tanaka, and Y. Oda, *Anal. Chem., 2009*, 81, 3836.
31. R. Yu, L. Xiao, G. Zhao, J. W. Christman, and R. B. van Breemen, *J. Pharmacol. Exp. Ther., 2011*, 339, 716.
32. D. Mangal, C. E. Uboh, and L. R. Soma, *Rapid Commun. Mass Spectrom.*, 2011, 25, 585.
33. M. Masoodi and A. Nicolau, *Rapid Commun. Mass Spectrom.*, 2006, 20, 3023.
34. W. Lin, M.-Q. Huang, X. Xue, L. Carter, N. A. Thomas, B. A. Bandeira-Melo, and K. Gemzell-Danielsson, *Anal. Biochem.*, 2014, 494, 25.
35. E. Ramadan, M. Basselin, J. S. Rao, L. Chang, M. Chen, K. Ma, and S. I. Rapoport, *Int. J. Neurpsychopharmacol., 2012*, 15, 931.