



PROVEN SOLUTIONS FOR LIPID ANALYSIS

Cayman's Historic Lipid Research Contributions

Page 1

Eicosanoid Analysis: Thoughts on Internal Standards and Reference Standards

Pages 2-5

Specialized Pro-Resolving Mediators and Eicosanoids: A Preferred Solid-Phase Extraction Protocol from Tissues and Biological Fluids

Pages 7-9

Lipidomics Services

Page 11

Resources for Lipid Researchers

Page 13

Common MRM Transitions

Page 14

40 YEARS OF SOLUTIONS

In the 1980s, Cayman introduced its very first products, naturally occurring prostaglandins isolated from gorgonian coral. Over the next decade, Cayman developed methods for synthetic production of prostaglandins and introduced the first commercial ELISA for prostaglandins and leukotrienes. Today, Cayman offers numerous ELISAs for a wide range of bioactive lipids, constantly improving on the reliability and sensitivity of these assays. More recently, Cayman has been leading efforts to develop mass spectrometry solutions for lipid analysis, including our expansive catalog of high-purity lipid standards.

1980

Founded by current CEO, Dr. Kirk Maxey, to demonstrate naturally growing gorgonian coral as a renewable, economically viable source of prostaglandins

1981

Extracted our very first product, PGA_2 , from gorgonian coral collected near Fisherman's Cay of the Cayman Islands

1982

Instituted methods for total synthesis of prostaglandins and developed immunoassays to detect steroids, lipids, and neuropeptides

1987

Introduced the first commercial ELISA for PGE_2

1991

Established a Protein Core and Antibody Development lab to support our evolving product lines

1996

Introduced an ELISA for 8-isoprostane as a relative indicator of antioxidant deficiency and oxidative stress

1998

Expanded operations of our Organic Chemistry department

2000

Developed an 8-isoprostane affinity sorbent for rapid sample purification

2003

Launched our Analytical Chemistry services using the enzyme activity and ELISA kits available in our catalog to test client samples

2006

Acquired a large-scale synthesis facility in the Czech Republic specializing in prostaglandin API production

Joined the LIPID MAPS® Consortium

2007

Offered the first resolvin product in our catalog, resolvin E1

2014

Synthesized a novel family of branched fatty acid hydroxy fatty acids (FAHFAs)

2015

Discovered and patented a series of EP_4 receptor agonists for potential osteogenic capacity

2016

Formed a dedicated Natural Products Chemistry department

2017

Established our LC-MS-based Targeted and Discovery Lipidomics services

2018

Introduced our trademarked line of lipid MaxSpec® standards and kits designed to simplify mass spectrometry workflows

2019

Expanded our polar lipid catalog by acquiring Matreya, LLC with strengths in sphingolipids and glycerophospholipids

2020

Lead EP_4 agonist KMN-159 to be tested as the API in collagen matrix for bone regrowth in an animal model of musculoskeletal injury

Eicosanoid Analysis: Thoughts on Internal Standards and Reference Standards

Robert C. Murphy¹ and Miguel Gijón²

¹University of Colorado Anschutz Medical Campus, Aurora, CO; ²Cayman Chemical Company, Ann Arbor, MI

A Brief History of Quantifying Bioactive Lipids

Over the past 50 years, mass spectrometry has emerged as the gold standard for the quantitative analysis of bioactive lipids, including metabolites of arachidonic acid. Significant advances in mass analyzer hardware and ionization processes have brought this technique to the forefront. The first quantitative studies of prostaglandins involved the use of a magnetic sector mass spectrometer or a single quadrupole mass analyzer coupled to a gas chromatograph, recording a small number of specific mass-to-charge ratios (m/z). In order to carry out these experiments, it was necessary to derivatize the prostaglandin to make it sufficiently volatile to pass through the gas chromatograph. Electron ionization imparted a large amount of internal energy to neutral molecules, resulting in intramolecular decomposition reactions and production of fragment ions, which were then monitored to specifically measure target molecules. The important principle realized from these early experiments was that the mass spectrometer was a remarkable quantitative tool when measuring ion abundances, but only if taking exquisite care of the experimental details. Specifically, the importance of an internal standard was immediately recognized.^{1,2} The internal standard corrected for the large number of instrumental variables that determined the mass spectrometer response and thus, the correlation between concentration of a molecular target and abundance of its fragment ions. The internal standard also helped identify analytes by chromatographic co-elution. Although various quantitation strategies could be employed, the most widely used were based on calibration curves to establish the relationship between the concentration of a target analyte and the measured ion abundance relative to that of the internal standard when prepared under identical isolation and derivatization steps.

Important changes have occurred just in the past 25 years, with the emergence of electrospray ionization (ESI) and remarkable developments in tandem mass spectrometry, including tandem quadrupole mass spectrometer systems and ion trap-based mass analyzers. ESI has eliminated the need to derivatize nonvolatile molecules, such as eicosanoids, while generating abundant carboxylate anions from most of these bioactive lipids. However, ESI is a

rather low-energy process, rarely yielding useful fragment ions. Both in tandem quadrupole and in ion trap mass spectrometers, this limitation is overcome through collision of ions with neutral gas molecules in order to increase the internal energy of precursor ions and initiate intramolecular rearrangement and fragmentation processes, resulting in product ions. The advantage of tandem mass spectrometry is to allow precise measurement of precursor-product ion relationships, adding important molecular signatures that strengthen the identification of the molecule. Another very important advancement has been the ability to drastically increase the number of precursor-product ion pair transitions that can be monitored during a single duty cycle (*i.e.*, the amount of time taken to monitor one complete series of transitions, repeated during the entire chromatographic run). At first, it was possible only to measure five to ten ion pairs in a duty cycle, but improvements in ion detector technology and fast-scanning electronic circuits have made it possible to measure tens to hundreds of ion transitions.

Stable Isotope-Labeled Internal Standards

The availability of stable isotope-labeled internal standards for eicosanoids has developed hand-in-hand with the advances in mass spectrometer instrumentation and ionization processes. Isotopic variants differ only in their molecular weight from unlabeled target eicosanoids. Such molecules behave in an identical manner to the naturally occurring molecules during sample preparation from the biological matrix. Any potential loss of eicosanoid is completely compensated by the loss of the stable isotope-labeled internal standard. A critical point is to add the internal standard as soon as possible to the sample, so that the ratio of endogenous eicosanoid to internal standard is established before any potential physical loss or chemical degradation. Once this ratio is set, it corrects for any problems associated with isolation and chromatography. The behavior of the stable isotope-labeled eicosanoid is identical to that of the unlabeled natural product with the exception of kinetic isotope effects. These effects are typically quite small even for deuterium-labeled analogs but become observable during separation by HPLC or capillary gas chromatography. Usually, the deuterated

analog precedes elution of the unlabeled species, most likely due to tighter carbon-deuterium bonds relative to carbon-hydrogen bonds, making the molecule somewhat smaller. Separation depends on the number of deuterium atoms in the standard, and one observes no effect in the chromatography of standards labeled with carbon-13 or oxygen-18.

A second important aspect of labeled internal standards is their behavior upon mass spectrometry analysis, in particular collision-induced dissociation. The m/z of the molecular ion will always be shifted by the excess mass of labeled atoms in the molecule, but the product ion may or may not be, depending upon the ion chemistry leading to its formation and the exact positions of the stable isotopes in the molecule. This behavior is also quite useful in trying to understand the mechanism by which ions arise in the collisional activation process.³

It is important to consider the total number of stable isotopes present in the molecule because of the occurrence of natural stable isotopes, in particular carbon-13, and to experimentally determine the population of isotope-labeled species in internal standards by calculating the atom percent excess over the natural abundance of carbon-13. While it is essential to know precisely the m/z of internal standard molecular and fragment ions in a quantitative

assay, one cannot assume that, for example, $\text{PGE}_2\text{-d}_4$ is 100% d_4 with no d_3 , d_2 , d_1 , or d_0 variants.

The abundance of the d_0 variant (*i.e.*, the unlabeled eicosanoid) is of great concern because it has an important influence on the standard curve generated. Standard curves are prepared by adding increasing amounts of reference standard to fixed amounts of labeled internal standard. We will use a theoretical example in which we add either $\text{LTB}_4\text{-d}_1$ (containing 10% $\text{LTB}_4\text{-d}_0$) or two different $\text{LTB}_4\text{-d}_4$ internal standard preparations (containing either 10% or 0.1% $\text{LTB}_4\text{-d}_0$) to a final 1 pM concentration in methanol/water, then add increasing concentrations (0.1 fM to 30 nM) of unlabeled LTB_4 (**Figure 1**). After injecting into a reversed-phase LC-MS/MS system, the abundance of ion transitions from m/z 335 ($\text{LTB}_4\text{-d}_0$) to m/z 196 or the abundance of ion transitions from m/z 339 ($\text{LTB}_4\text{-d}_4$) to m/z 197 are determined (assuming $[\text{5-d}_1]\text{LTB}_4$ and $[\text{6,7,14,15-d}_4]\text{LTB}_4$ internal standards, respectively). In this experiment, the three different internal standards illustrate the influence that isotope content has on the calibration curve dynamic range and the asymptotic lines where the ratio of LTB_4 to internal standard becomes constant. In order to carry out the quantitation, it is necessary to take into account the deuterated isotope content of the three different labeled LTB_4 preparations, as well as the naturally

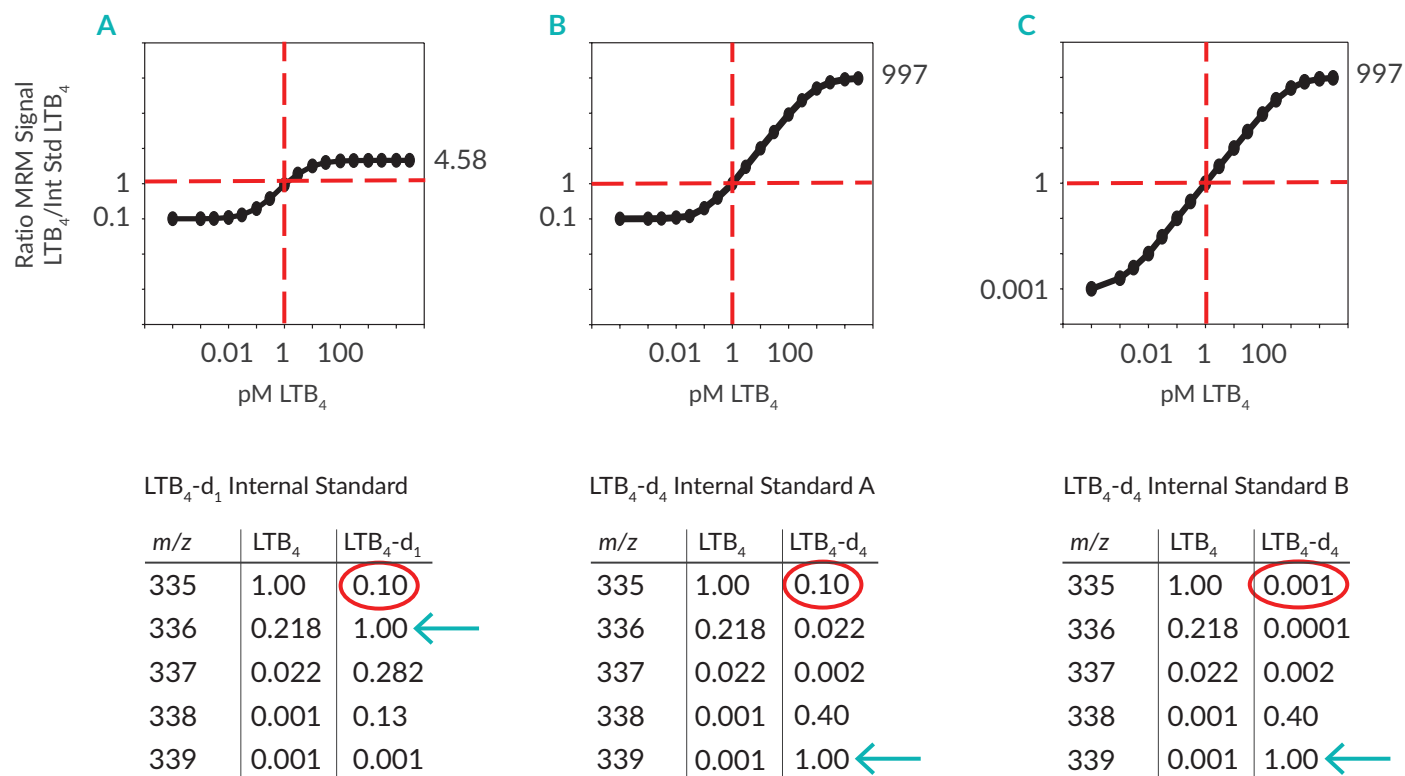


Figure 1. Theoretical standard curves for the quantitation of LTB_4 adding equal amounts (1 pM) of three different deuterium-labeled internal standards to various quantities of LTB_4 in solution (0.0001 to 30,000 pM). The signals from LTB_4 using negative-ion electrospray mass spectrometry were measured from m/z 335 to m/z 339. A. Internal standard having only one deuterium atom and containing 10% unlabeled LTB_4 . B. Internal standard having four deuterium atoms and containing 10% unlabeled LTB_4 . C. Internal standard having four deuterium atoms and containing 0.1% unlabeled LTB_4 . The tables under each graph show the measured isotopic abundance of the different ions relative to the molecular ion of the internal standard (arrows).

occurring carbon-13 content. As seen in **Figure 1A**, when 10% $\text{LTB}_4\text{-d}_0$ (m/z 335) is in the $\text{LTB}_4\text{-d}_1$ internal standard, the standard curve has a very narrow dynamic range in that it is linear only over a very small portion of ratios m/z 335/336. The useful range is only 0.3 to 10 pM. Dynamic range is considerably increased when using $\text{LTB}_4\text{-d}_4$, even with 10% $\text{LTB}_4\text{-d}_0$ present (**Figure 1B**). Specifically, this is because the probability of LTB_4 containing four carbon-13 atoms is very low. The useful range of this standard curve is 0.03 to 100 pM. The theoretical ratio of analyte-to-internal standard becomes constant at 0.1 pM, at the lower end of the curve for both internal standards. When the content of $\text{LTB}_4\text{-d}_0$ is reduced to 0.1%, a much wider dynamic range is observed from 0.03 to 100 pM (**Figure 1C**). Notice that the intermediately labeled species (e.g., $\text{LTB}_4\text{-d}_3$ at 40% in this example) has no effect on the standard curve whatsoever. In general, the overall amount of internal standard added to samples is rather immaterial as long as it is held constant. Precise addition is critical, though.

In general, standard curves using a stable isotope-labeled internal standard are sigmoid, with two asymptotic regions. The region at the left is driven by the isotopic purity of the internal standard at the molecular weight of the eicosanoid, while the right side of the asymptotic region corresponds to the natural abundance of carbon-13 and oxygen-18 at the molecular weight of the labeled standard (**Figure 2**). Since standard curves are most often employed for small

amounts of eicosanoids, the total number of atoms increasing the mass of the internal standard becomes most important. Thus, to achieve maximum dynamic range of an analytical assay it is critical to optimize hydrogen-deuterium exchange chemistry or incorporation of oxygen-18 in the carboxylate moiety of standards by either chemical or enzymatic means⁴ to minimize the presence of unlabeled material.

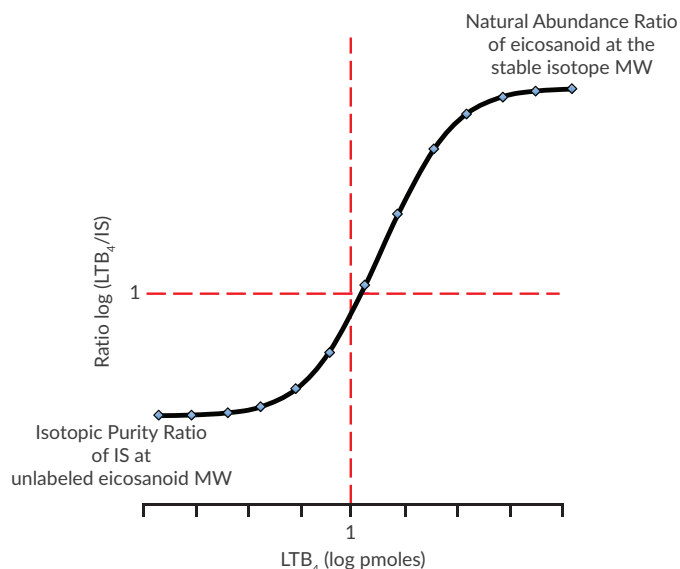


Figure 2. Theoretical standard curve for stable isotope dilution of LTB_4 over a large dynamic range, indicating the asymptotic regions at the limits of the assay which are influenced by the total mass shift (number of stable isotopes) of the internal standard (IS) and the isotopic purity.

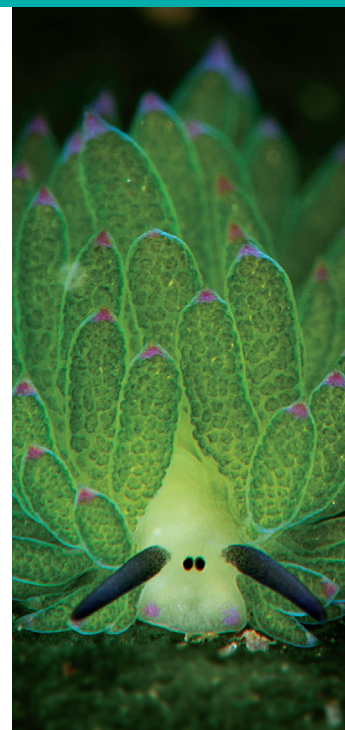
ISOTOPICALLY LABELED STANDARDS

Cayman offers more than 250 isotopically enriched lipid molecules that enable confidence in quantifying analytes of interest. This includes deuterium-labeled (^2H) standards manufactured to contain less than 1% of unlabeled (d_0) molecules as well as ^{13}C -labeled standards. If you cannot find an internal standard fit for the methods you are performing, contact us for a custom synthesis quote.

More Than 250 Internal Standards Available for:

- Prostaglandins
- Fatty Acids
- Sterol Lipids
- Sphingolipids
- Octadecanoids
- Fatty Amides
- Fatty Esters/Ethers
- Docosanoids
- Glycerophospholipids
- And More

Find the right internal standard using the “Isotopically Labeled Standards” search facet to filter by ^{13}C , ^{15}N , or deuterium labels on caymanchem.com



Reference Standards

Often overlooked is the importance of reference standards in performing quantitative assays. The purity of the reference standard establishes the accuracy of the method. Of course, if the reference standard is a sodium or ammonium salt of the carboxylate anion, it needs to be taken into account when calculating the molarity of the standard dilutions, but the presence of unknown impurities will also lead to errors in accuracy. Oftentimes, different levels of eicosanoids are reported between laboratories, in part because of varying purities of the reference standards used. Unfortunately, there is no convenient way to assess the quantity of a prostaglandin or an unsaturated fatty acid except for gravimetric measurements. This is not the case for leukotrienes or HETEs, where one can employ Beer's law to calculate the concentration of a solution based upon UV absorption and known molar extinction coefficients of these conjugated olefins.



The purity of the reference standard establishes the accuracy of the method.

It is also important to carefully store any reference standard solutions, since instability of any of the eicosanoids, frequently due to oxidation, would lead to errors when generating the standard curve. This is not as critical for stable isotope-labeled solutions. As long as one prepares the standard curve for each batch of analyses, the quantity of isotope-labeled internal standard added to each sample is invariant, even though it may not be accurately known. Of course, it is still essential to ensure that no oxidation or degradation products of these standards interfere with any of the ion transitions being measured.

In summary, continuing advances in mass spectrometry instruments and increasing availability of high-quality analytical standards are allowing the accurate quantitation of eicosanoids by scientists around the world. As new lipid mediators keep being discovered, it is an ongoing challenge to maintain the availability of adequate tools for the study of these molecules and their roles in physiology and disease.

Article References

1. Sweeley, C.C., Elliott, W.H., Fries, I., et al. Mass spectrometric determination of unresolved components in gas chromatographic effluents. *Anal. Chem.* **38**(11), 1549-1553 (1966).
2. Samuelsson, B., Hamberg, M., and Sweeley, C.C. Quantitative gas chromatography of prostaglandin E₁ at the nanogram level: Use of deuterated carrier and multiple-ion analyzer. *Anal. Biochem.* **38**(1), 301-304 (1970).
3. Murphy, R.C., Barkley, R.M., Zemski Berry, K., et al. Electrospray ionization and tandem mass spectrometry of eicosanoids. *Anal. Biochem.* **346**(1), 1-42 (2005).
4. Murphy, R.C. and Clay, K.L. Preparation of ¹⁸O derivatives of eicosanoids for GC-MS quantitative analysis. *Methods Enzymol.* **86**, 547-551 (1982).



About the Authors



Robert C. Murphy, Ph.D.

Dr. Murphy is an Emeritus Professor in the Department of Pharmacology at the University of Colorado Anschutz Medical Campus. He has dedicated much of his life to the study of bioactive lipids, largely using and developing sophisticated mass spectrometry techniques. By elucidating the structure of Slow-Reacting Substance of Anaphylaxis (SRS-A), which he termed leukotriene C₄, he forged novel avenues for research on a unique pathway of arachidonic acid metabolism. He has mentored many scientists and influenced careers. He has received numerous awards throughout his own career, including his election as President of the American Society for Mass Spectrometry and serving on its Board of Directors.



Miguel Gijón, Ph.D.

Dr. Gijón is a scientist at Cayman Chemical. His career interests, sparked by the study of lipid mediators of inflammation, include the biological roles of lipids in disease, the catalytic mechanisms and regulation of enzymes implicated in lipid metabolism, and the detailed description of lipid composition in cells and tissues. He is currently a key member of the lipidomics services team, developing or adapting lipid extraction and mass spectrometry-based analysis protocols, as well as discussing experimental models with other researchers to find the most useful approaches to their lipid analysis needs. He maintains active collaborations with academic scientists.



LIPID REFERENCE STANDARDS

Cayman is a world leader in the synthesis, purification, and characterization of lipids with 40 years' experience. We are committed to the development and manufacture of lipids and biochemicals of the highest quality and value. Our experience in chemical synthesis and in the extraction and purification of natural products allows us to achieve the best attainable purity using state-of-the-art techniques.

We produce an extensive collection of lipids that can be used as research standards in biotechnology and pharmaceutical pursuits. Many are formulated in small quantities or in solution for ease of use in mass spectrometry analysis. With Cayman lipids, you can be confident that you will receive highly pure compounds that have passed our internal QC characterization tests, as well as world-class technical support backed by our team of in-house chemists.

More Than 2,300 Lipids Available for:

Fatty Acyls

- Eicosanoids
- Docosanoids
- Fatty Acids
- Fatty Acid Conjugates
- Fatty Alcohols/Aldehydes
- Fatty Amides
- Fatty Esters/Ethers
- Hydrocarbons
- Octadecanoids

Glycerolipids

- Monoradylglycerols
- Diradylglycerols
- Triradylglycerols

Glycerophospholipids

- Glycerophosphocholines (PC)
- Glycerophosphoethanolamines (PE)
- Glycerophosphoserines (PS)
- Glycerophosphoglycerols (PG)
- Glycerophosphoinositols (PI)
- Glycerophosphates (PA)
- Oxidized Glycerophospholipids

Prenol Lipids

- Isoprenoids
- Quinones/Hydroquinones
- Polyprenols

Sphingolipids

- Ceramides
- Glycosphingolipids
- Sphingoid Bases
- Sphingomyelins

Sterol Lipids

- Bile Acids
- Steroids
- Sterols

As a gold standard for lipid categorization, Cayman follows the LIPID MAPS® classification system.

Browse our extensive and continuously expanding line of lipid standards using the "Lipids" search facet on caymanchem.com

Specialized Pro-Resolving Mediators and Eicosanoids: A Preferred Solid-Phase Extraction Protocol from Tissues and Biological Fluids

Charlotte C. Jouvene and Charles N. Serhan

Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Following infection and/or injury, the acute inflammatory response is a protective mechanism initiated by the host. Ideally, complete resolution of inflammation allows a return to homeostasis.¹ Lipid mediators have crucial roles in both initiation of inflammation and its timely resolution. The cardinal signs of inflammation are initiated by specific eicosanoids, e.g., prostaglandins and leukotrienes (**Figure 1**), stimulating responses such as neutrophil recruitment. As a reflection of the neutrophil-monocyte sequence, a lipid mediator class switching occurs with the biosynthesis of specialized pro-resolving mediators (SPMs).² These SPMs include arachidonic acid (AA)-derived lipoxins (LX), eicosapentaenoic acid (EPA)-derived E-series resolvins (RvE), and docosahexaenoic acid (DHA)-derived D-series resolvins (RvD), protectins (PD), and maresins (MaR) that each limit neutrophil tissue infiltration and stimulate

non-phlogistic monocyte recruitment, allowing complete resolution of inflammation and a return to homeostasis (**Figure 1**).^{2,3} These potent mediators of resolution represent a challenge for quantitative extraction, notably due to their fragile physical properties and their picogram to nanogram bioactive concentration ranges in tissues.^{2,3}

Liquid-liquid extraction (LLE), one of the most widely used lipid extraction strategies, involves the use of immiscible organic solvents to extract phospholipids, fatty acids, triacylglycerols, etc.^{4,5} An often-used method using a mix of chloroform, methanol, and water was introduced by Folch *et al.*^{6,7} and modified by Bligh and Dyer (**Figure 2**).⁸

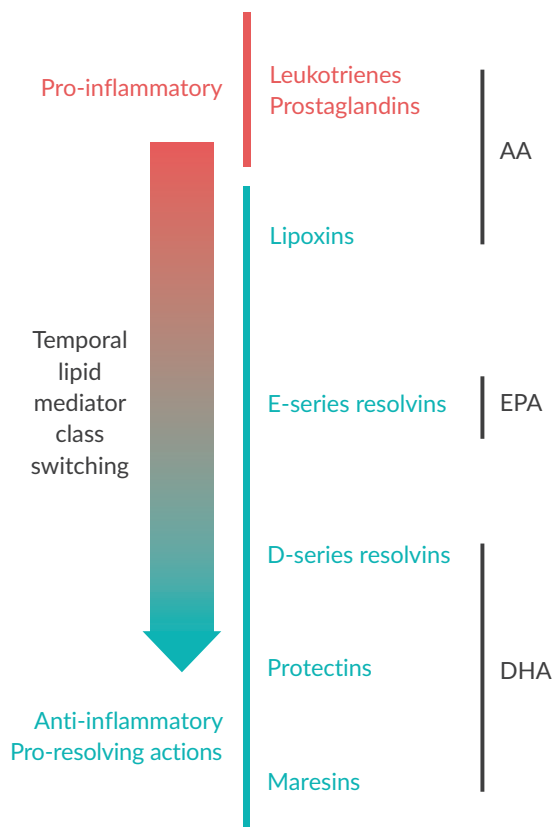


Figure 1. Inflammation-resolution time course: lipid mediator class switching during inflammatory processes. Pro-inflammatory prostaglandins and leukotrienes are biosynthesized from arachidonic acid during initiation of inflammation. Later, pro-resolving mediators (lipoxins, E-series resolvins, D-series resolvins, protectins, and maresins) are actively biosynthesized during the resolution phase.

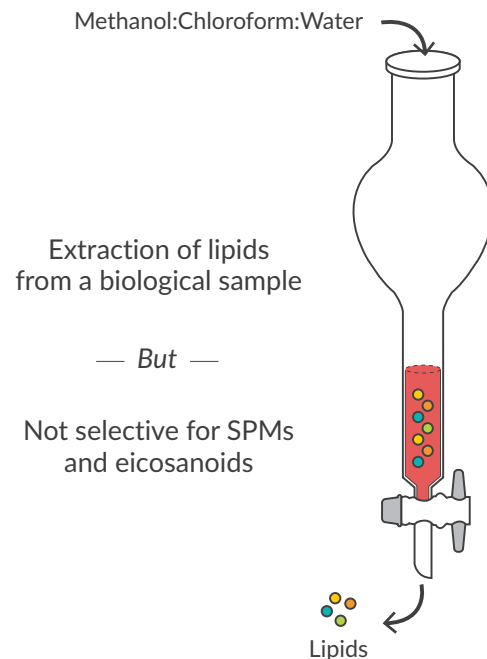
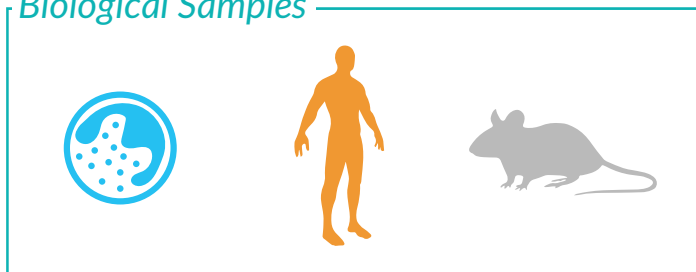


Figure 2. Liquid-Liquid Extraction

Although effective for phospholipids and fatty acid extraction, this method does not achieve selective extraction of eicosanoids and SPMs. Selective retention of eicosanoids and SPMs can be done by their different interactions between a solid phase and a liquid mobile phase during solid-phase extraction (SPE) (see outline of the current procedures used by our laboratory, **Figure 3**). Moreover, SPE is preferred over traditional LLE because it is a rapid procedure that uses less solvent and is more selective.⁹

SPM and Eicosanoid Extraction Procedure

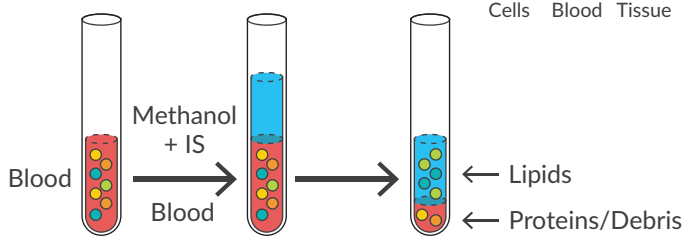
Biological Samples



Sample Preparation & Protein Precipitation

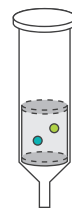
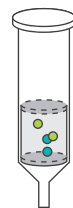
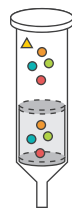
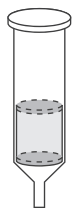
+ Methanol
↳ Protein precipitation

+ Deuterated Internal Standards (IS)
↳ Identification and quantification



Solid-Phase Extraction

1. Conditioning
• Methanol
• Water
2. Loading
• Water, pH 3.5
(with <10% methanol)
3. Washing
• Water
• Hexane
4. Elution
• Methyl formate



Steps for Maximal SPM Recovery

(Note this procedure is tissue-dependent and routinely gives >85-95% recovery for IS that are ideally deuterium-labeled.)

1. Add IS-containing ice-cold methanol to sample. Methanol enables the separation of lipids from proteins after homogenization, and appropriate IS are used for the identification and the quantification of SPMs and eicosanoids (see tinyurl.com/spectrabook2019).¹⁰
 2. Keep tissues or biological fluids at -20°C for 45 min to allow protein precipitation.
 3. After centrifugation (1,000 x g, 10 min, 4°C), if necessary, bring the sample volume down to ~1 ml with a gentle stream of nitrogen.
 4. Extract lipid mediators by automatic SPE (Extrahera™, Biotage®):
 - Condition the C18 columns (Isolute® SPE 100 mg, Biotage®) used for SPE with 5-10 ml* methanol and 5-10 ml* water. Other C18 columns such as Bond Elut C18 (Agilent) or Sep-Pak C18 (Waters) can be used.
 - Load the samples (water pH 3.5/methanol 9:1).
 - Rapidly (<30 sec) wash the column with 5-10 ml* water to return to an apparent neutral pH ~7.0 (to be tested before extraction). This step reduces acid-induced isomerization, loss of analytes, and lactone formation, e.g., 5-HETE, etc.
 - Wash with hexane to elute more polar lipids.
 - Elute SPMs and eicosanoids with 5-10 ml* of methyl formate.
- *Optimized for 30 mg of tissue.
5. Remove the solvent with a gentle stream of nitrogen.
 6. Resuspend in methanol/water (50:50) prior to LC-MS/MS injection.

LC-MS/MS

Identification and Quantification

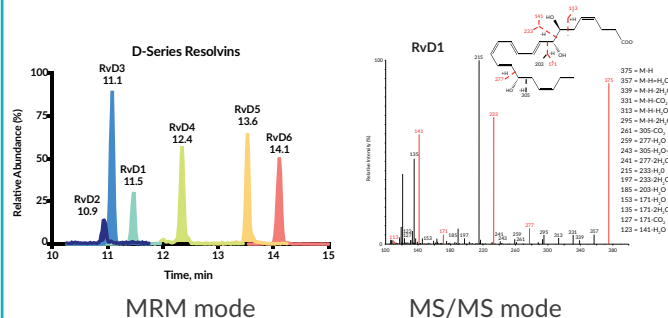


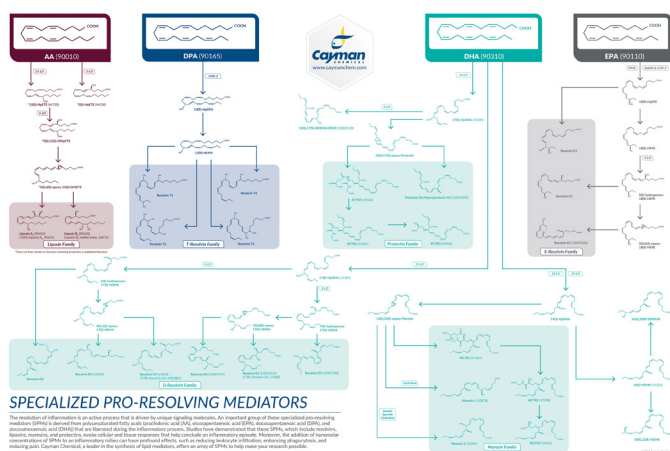
Figure 3. Steps to extract SPMs effectively from a tissue or a biological fluid. As a precaution, tissues and/or biological fluids should either be directly prepared for the extraction after harvesting or rapidly snap frozen before storage at -80°C to reduce potential autoxidation and hydrolysis. Samples should always be kept on ice to prevent isomerization of the lipid mediators.

REQUEST OUR PATHWAY POSTER

Specialized Pro-Resolving Mediators

As a leader in the synthesis of lipid mediators, Cayman offers many of the key SPMs to aid in a better understanding of these unique signaling molecules.

This wall poster details the biosynthesis of SPMs from polyunsaturated fatty acids (AA, EPA, DPA, and DHA) that are liberated during the inflammatory process. The poster also includes Cayman item numbers for many of these compounds that are available as reference standards.



Request a copy of this poster and explore others in the Lipid Biochemistry series at caymanchem.com/literature

Additional Lipid Wall Posters from Cayman

- Arachidonic Acid Cascade
- Cannabinoid Receptors
- Eicosanoid GPCRs
- Lysophospholipid Signaling

Good handling and appropriate conditions for the sample extractions are as important as the LC-MS/MS settings used for lipid mediator analysis. Detailed information can be found in these references.^{4,5,10-13}

Article References

1. Ward, P.A., Acute and chronic inflammation. *Fundamentals of inflammation*. Serhan, C.N., Ward, P.A., and Gilroy, D.W., editors. 1st edition, Cambridge University Press (2010).
2. Serhan, C.N. Pro-resolving lipid mediators are leads for resolution physiology. *Nature* **510(7503)**, 92-101 (2014).
3. Serhan, C.N. Discovery of specialized pro-resolving mediators marks the dawn of resolution physiology and pharmacology. *Mol. Aspects Med.* **58**, 1-11 (2017).
4. Gunstone, F.D., Harwood, J.L., and Padley, F.B. *The Lipid Handbook*, Second Edition, Chapman and Hall, London, (1994).
5. Okuno, T. and Yokomizo, T. Basic techniques for lipid extraction from tissues and cells. *Bioactive Lipid Mediators: Current reviews and protocols*. Yokomizo, T. and Murakami, M, editors. 1st edition, Springer (2015).
6. Folch, J., Ascoli, I., Lees, M., et al. Preparation of lipide extracts from brain tissue. *J. Biol. Chem.* **191(2)**, 833-841 (1951).
7. Folch, J., Lees, M., and Sloane Stanley, G.H. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226(1)**, 497-509 (1957).
8. Bligh, E.G. and Dyer, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37(8)**, 911-917 (1959).
9. Powell, W.S. Extraction of eicosanoids from biological fluids, cells, and tissues. *Methods Mol. Biol.* **120**, 11-24 (1999).
10. Riley, I., Norris, P.C., Luong, K., et al. CET&RI Spectra Book & Physical Properties of SPM and Eicosanoids 2019, tinyurl.com/spectrabook2019.
11. Dalli, J., Colas, R.A., Walker, M.E., et al. Lipid mediator metabolomics via LC-MS/MS profiling and analysis. *Methods Mol. Biol.* **1730**, 59-72 (2018).
12. English, J.T., Norris, P.C., Hodges, R.R., et al. Identification and profiling of specialized pro-resolving mediators in human tears by lipid mediator metabolomics. *Prostaglandins Leukot. Essent. Fatty Acids* **117**, 17-27 (2017).
13. Norris, P.C. and Serhan, C.N. Metabolomic profiling of functional immunoresolvent clusters and eicosanoids in mammalian tissues. *Biochem. Biophys. Res. Commun.* **504(3)**, 553-561 (2018).

About the Authors

CJ **Charlotte C. Jouvene, Ph.D.**
Dr. Jouvene obtained her degree in biochemistry in France and is currently a post-doctoral fellow and visiting scientist at the Center for Experimental Therapeutics and Reperfusion Injury (CET&RI) at Brigham and Women's Hospital and Harvard Medical School.

CS **Charles N. Serhan, Ph.D., D.Sc.**
Charles N. Serhan is Professor and Director of the CET&RI at Brigham and Women's Hospital. He is also the Simon Gelman Professor of Anesthesia (Biochemistry & Molecular Pharmacology) at Harvard Medical School and Professor of Oral Medicine, Infection and Immunity at Harvard School of Dental Medicine.

Learn more about the Serhan Lab and find links to protocols and publications at <http://serhanlab.bwh.harvard.edu>

Ready-to-Use, Quantitative Standards

Cayman has translated its vast expertise in the synthesis, purification, and characterization of lipids into our line of MaxSpec[®] standards designed to simplify mass spectrometry workflows. MaxSpec[®] standards are gravimetrically prepared in solution and supplied in glass ampules to save time in the preparation of standards and samples.

In addition to providing ease of use, MaxSpec[®] standards are characterized with a defined concentration as well as by purity and identity tests to enable quantitative accuracy and reproducibility.

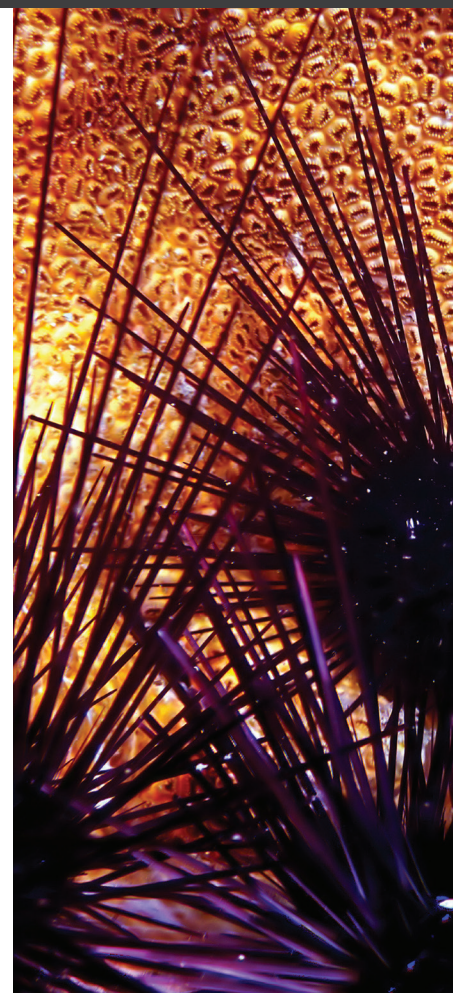
- ✓ Verified concentration
- ✓ LC-MS identity test
- ✓ HPLC purity test
- ✓ Ongoing stability testing



MaxSpec[®] Standards Now Available for Key SPMs:

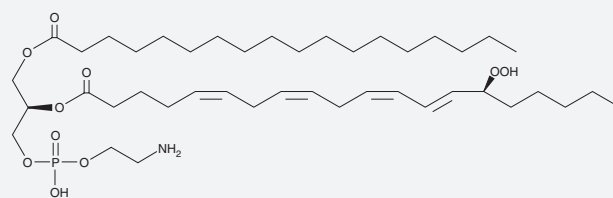
Lipoxin A₄, Lipoxin A₄-d₅, Maresin 1-d₅, and Resolvin D1

Explore MaxSpec[®] Analytical Standards for prostaglandins, docosanoids, fatty acids, and more at [caymanchem.com/maxspec](https://www.caymanchem.com/maxspec)



NEW: OXIDIZED PHOSPHOLIPID STANDARDS

Cayman is the first commercial supplier of novel oxidized phospholipids, which contain either a hydroxy or hydroperoxy group on the *sn*-2 fatty acid side chain. Oxidized phospholipids are found in the membranes of activated immune cells and platelets and serve as critical biomarkers of ferroptosis. These standards were used in a recent collaborative study between the University of Michigan and Cayman Chemical to show that an increase in oxidized phospholipids and ferroptosis occurs in response to cancer immunotherapy.



1-Stearoyl-2-15(S)-HpETE-*sn*-glycero-3-PE
Item No. 25856

Read the Publication Using these Standards:

CD8⁺ T cells regulate tumour ferroptosis during cancer immunotherapy

Nature **569**, 270–274 (2019)

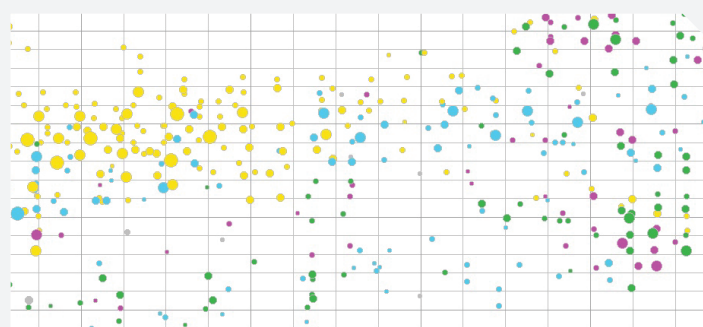
LIPIDOMICS SERVICES

Our lipid experts have decades of experience in lipid synthesis, purification, and characterization with access to state-of-the-art equipment for discovery and targeted lipidomics.

Discovery Lipidomics

Using an untargeted approach, our team can create a detailed lipid profile from thousands of lipid species or identify profile changes within a single lipid class.

- Fatty Acyls
- Glycerolipids
- Glycerophospholipids
- Sphingolipids
- Sterol Lipids



2D feature plot (m/z versus retention time) generated using the Lipostar lipidomics software package reveals different lipid classes in adipose tissue.

Targeted Lipidomics

Already know your lipid species of interest? Our team can perform targeted lipidomics on a known collection of lipids utilizing our standardized protocols and established LC-MS/MS methods. Our approach can quantify lipids present at low levels in samples and reveal changing trends in lipid species amongst experimental groups using sophisticated data analysis software.



Heat map generated from relative quantitation by LC-MS/MS of oxylipins.

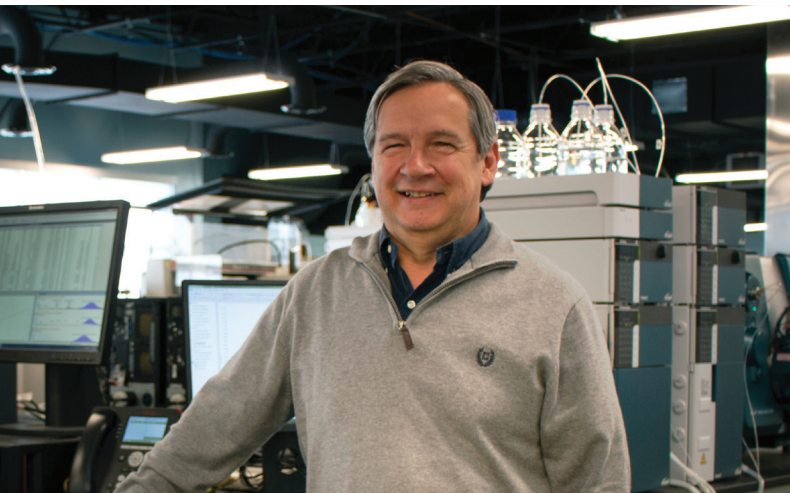
Targeted Lipid Panels

Maximize data from precious samples with lipid panels offering the sensitivity and specificity needed to efficiently identify and quantify biologically relevant lipids. Choose from the panels below or contact us to discuss a customized panel for your next project.

- Eicosanoids
- Oxidized Phospholipids
- Short-Chain Fatty Acids
- Endocannabinoids
- Cholesterol Esters
- Sphingolipids
- Urinary Lipid Metabolites

Learn more about our Lipidomics Services at caymanchem.com/lipidomics

Meet the Expert: Lipidomic Profiling Services



Dr. Miguel Gijón, Staff Scientist on Cayman's Bioanalytical Chemistry Team

You have put together a panel of deuterated internal standards and non-deuterated calibration standards including odd-chain fatty acids for quantitation of various lipids using LC-MS/MS. Describe how you developed these panels and the relevancy of analytes chosen. How can these panels be customized to a particular experiment?

We have begun with a set of standards for the analysis of oxylipins such as prostaglandins and leukotrienes, a natural fit for Cayman, and we have included many of the analytes that are best known in the relevant literature to be biologically active in a wide variety of models, particularly in promoting inflammation or the resolution of inflammation. The list of analytes can be easily customized according to each experiment, simply by modifying the list of mass-to-charge (m/z) transitions analyzed by the mass spectrometer and substituting the necessary internal and calibration standards. Cayman is starting to manufacture and distribute a new line of high-quality standards, labeled MaxSpec[®], in which the concentration, purity, and stability are guaranteed, thus ensuring reproducible and accurate quantitation.

How does the instrumentation available in your lab impact the types of services you offer?

Our lab houses state-of-the-art Triple quad and Orbitrap instruments, giving us the sensitivity, dynamic range, and analytical flexibility to provide qualitative (relative) and fully quantitative data to our customers in targeted experiments. If required, we also have the instrumentation to generate high-resolution spectra, which is extremely useful for distinguishing between closely related isobaric molecular species, such as identification of unknown analytes in untargeted experiments.

What opportunities do you see for growth in the field of Lipidomics?

I believe it is fair to state that Lipidomics is still in its infancy, so the potential for growth is immense. Improvements can be made in sensitivity, which will be highly beneficial since many bioactive lipids are present at very low levels in biological samples. As of today, analysis of certain lipids requires prior separation from other lipids that are much more abundant and can completely mask the signals for the minor components. Developing extraction strategies that will allow for the simultaneous analysis of different classes of molecules remains a major challenge. Another area of potential growth is the development of techniques that can be used in combination with current LC-MS or LC-MS/MS approaches to further define the molecular species analyzed (e.g., the position and configuration of double bonds in the acyl chains of phospholipids and glycerolipids). Finally, a critical feature of lipid mediators is that they are produced at specific locations within tissues and cells. Promising results in lipid mass spectrometry imaging will hopefully be expanded to add a spatial/compartamental dimension to the data, and eventually become one more technique in the Lipidomics toolbox.

Read the full interview at [caymanchem.com/lipidomics-expert](https://www.caymanchem.com/lipidomics-expert)

Resources for Lipid Researchers

Websites

[Advice from Lipid Experts at Cayman](#)

Cayman scientists have gathered their knowledge and advice about enzymology and metabolism, analytical techniques, sample handling and storage, and more as a resource page.

[Lipid Calculator](#)

This mass spectrometry tool calculates exact mass and abundance by enabling a search *via* lipid class, types of acyl groups, ESP ion polarity, and *m/z*.

[LIPID MAPS® Lipidomics Gateway](#)

This gateway provides access to lipid nomenclature, relational databases, tools for lipid drawing and calculation of lipid parameters, analysis protocols, standards, tutorials, publications, and other resources, including Bill Christie's encyclopedia, the LipidWeb. LIPID MAPS® preserves the largest internationally recognized classification system and public lipid-only structure database in the world.



[Lipidomics Standards Initiative](#)

This website offers guidelines for the major lipidomic workflows, including sample collection and storage as well as data deconvolution and reporting.

[International Lipidomics Society](#)

This non-profit organization promotes lipidomics by fostering the development of new technologies, techniques, resources, skills, and training.



[ASBMB Lipid Corner](#)

As the Lipid Research Division of the American Society for Biochemistry and Molecular Biology, this website supports the lipid community by highlighting research, offering a forum for ongoing discussions, spotlighting members, and maintaining a job board.

[The Lipid Web](#)

This website introduces the chemistry and biochemistry of individual lipid classes, along with a compendium of information on mass spectrometry of fatty acids.

[Cyberlipid Center](#)

This site from a non-profit scientific organization features an encyclopedia and annotated bibliography devoted to the study of all aspects of lipidology, including forgotten studies of the past, work in progress, and hot research topics.

[The Probes & Drugs Portal](#)

This portal is a public resource joining together focused libraries of bioactive compounds (e.g., probes, drugs, specific inhibitor sets, etc.) with commercially available screening libraries.



Databases

[mzCloud](#)

This extensively curated mass spectral database of high-resolution tandem mass spectra is arranged into spectral trees. It is a fully searchable library that allows spectra searches, tree searches, structure and substructure searches, monoisotopic mass searches, peak (*m/z*) searches, precursor searches, and name searches.

[METLIN](#)

This MS/MS database has multiple searching capabilities including single, batch, precursor ion, neutral loss, accurate mass, and fragment searches. It was generated at multiple collision energies and in positive and negative ionization modes using multiple instrument types including SCIEX, Agilent, Bruker, and Waters QTOF mass spectrometers.

Common MRM Transitions for Eicosanoids and Docosanoids (Negative ESI)

Analyte	Precursor Ion	Product Ion
AA	303	259
5(6)-DiHET	337	145
8(9)-DiHET	337	127
11(12)-DiHET	337	167
14(15)-DiHET	337	207
5(6)-DiHETE	335	145
14(15)-DiHETE	335	207
17(18)-DiHETE	335	247
DHA	327	229
DPA	329	231
16(17)-EpDPA	343	274
19(20)-EpDPA	343	299
8(9)-EpETE	317	123
11(12)-EpETE	317	179
14(15)-EpETE	317	207
17(18)-EpETE	317	215
5-OxoETE	317	129
15-OxoETE	319	113
5(6)-EET	319	191
8(9)-EET	319	155
11(12)-EET	319	167
14(15)-EET	319	219
EPA	301	257
4-HDHA	343	101
7-HDHA	343	141
8-HDHA	343	109
10-HDHA	343	153
11-HDHA	343	121
13-HDHA	343	193
14-HDHA	343	205
16-HDHA	343	233

Analyte	Precursor Ion	Product Ion
17-HDHA	343	245
20-HDHA	343	241
5-HEPE	317	115
8-HEPE	317	155
9-HEPE	317	149
11-HEPE	317	167
12-HEPE	317	179
15-HEPE	317	219
18-HEPE	317	259
5-HETE	319	115
8-HETE	319	155
9-HETE	319	151
11-HETE	319	167
12-HETE	319	179
15-HETE	319	219
16-HETE	319	233
17-HETE	319	247
18-HETE	319	261
19-HETE	319	231
20-HETE	319	289
LTB ₄	335	195
20-carboxy LTB ₄	365	201
20-hydroxy LTB ₄	351	195
LTC ₄	624	272
LTD ₄	495	143
LTE ₄	438	333
LXA ₄	351	115
Maresin 1	359	177
PGB ₂	333	235
PGB ₃	331	269
PGD ₂	351	271

Analyte	Precursor Ion	Product Ion
PGD ₃	349	269
PGE ₂	351	271
PGE ₃	349	269
2,3-dinor-6-keto PGF _{1α}	341	135
6 keto PGF _{1α}	369	163
PGF _{2α}	353	193
PGF _{3α}	351	193
PGJ ₂	333	189
15-deoxy PGJ ₂	315	271
Resolvin D1	375	215
Resolvin D2	375	175
TXB ₂	369	169
11-dehydro TXB ₂	367	305
2,3-dinor TXB ₂	341	137
TXB ₃	367	169

Deuterated Analyte	Precursor Ion	Product Ion
AA-d ₈	311	267
DHA-d ₅	332	288
11(12)-DiHET-d ₁₁	348	167
8(9)-EET-d ₁₁	330	155
EPA-d ₅	306	262
5-HETE-d ₈	327	309
20-HETE-d ₆	325	281
LTB ₄ -d ₄	339	197
PGE ₂ -d ₄	355	319
6-keto PGF _{1α} -d ₄	373	167
PGF _{2α} -d ₄	357	197
TXB ₂ -d ₄	373	173
11-dehydro TXB ₂ -d ₄	371	309

Find additional lipid resources including advice from lipid experts, wall posters, and software at caymanchem.com/lipidresources



1180 East Ellsworth Road
Ann Arbor, MI 48108
www.caymanchem.com

CONTACT US

PHONE:

(800) 364-9897 (USA and Canada only)
(734) 971-3335

FAX:


(734) 971-3640

EMAIL:

Sales: sales@caymanchem.com
Customer Service: custserv@caymanchem.com
Technical Support: techserv@caymanchem.com
Contract Services: contractresearch@caymanchem.com

SOCIAL:

 www.facebook.com/caymanchemical

 [@CaymanChemical](https://twitter.com/CaymanChemical)

Distributed by:



BIOMOL GmbH
Kieler Str. 303a
22525 Hamburg

www.biomol.de
Tel: 040-8532600
Fax: 040-85326022
info@biomol.de

Toll-free in Germany:
Tel: 0800-2466651
Fax: 0800-2466652