Technical recommendations for analyzing oxylipins by liquid chromatography–mass spectrometry

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Abstract

Several oxylipins are potent lipid mediators that regulate diverse aspects of health and disease and whose quantitative analysis by liquid chromatography–mass spectrometry (LC-MS) presents substantial technical challenges. As members of the lipidomics community, we developed technical recommendations to ensure best practices when quantifying oxylipins by LC-MS.

Lipid signaling mediators are essential factors in health and disease, participating in diverse cellular processes related to inflammation, immunity, development, and homeostasis. A major category of lipid mediators, comprising large families of structurally related fatty acyls, are oxylipins, which include eicosanoids such as prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs) and epoxyeicosatrienoic acids (EETs), all of which are derived from arachidonic acid. Other oxylipins are derived from shorter- or longer-chain polyunsaturated fatty acids (PUFAs), such as octadecanoids or docosanoids, including specialized pro-resolving mediators (SPMs). Whereas most oxylipins are generated by enzymes such as lipoxygenases (LOXs), cyclooxygenases (COXs), and cytochrome P450 monooxygenases (CYPs), they can also be formed nonenzymatically by autoxidation. The relevance of oxylipins to human disease is undisputed. For example, well-known drugs target the prostaglandin pathway to modulate inflammatory diseases, including nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and diclofenac. In addition, aspirin, which blocks thromboxane biosynthesis when used at low doses, is the most widely prescribed drug globally, with a major role in the secondary prevention of cardiovascular diseases and emerging potential in reducing cancer incidence (*1*).

Eicosanoids derived from arachidonic acid were first discovered in the 1930s by von Euler (*2*). This was followed by the structural characterization of PGs and TXs in the second half of the 20th century, leading to the awarding of the Nobel Prize to Bergström and Samuelsson in 1982 (*3*), together with Vane for discovering the mechanism of action of aspirin (*4*). Since then, additional families were found, and key mechanisms of oxylipin signaling, metabolism, and excretion in urine were revealed in numerous biological and pathophysiological contexts. After their initial discovery, oxylipins were often named based on their cellular source; for example, prostaglandins were first identified in seminal vesicles. Alternatively, they were named based

on a combination of source and chemical structure; for example, with LT being made by white blood cells and carrying a triene motif. Although oxylipins are often described as lipid mediators or autacoids that are secreted by cells to act on receptors locally, the biological functions of many oxylipins are still to be established. Only some, for example, PGs and LTs, have G protein–coupled receptors that are formally validated by the International Union of Pharmacology (IUPHAR). In the LIPID MAPS classification (*5*), oxylipins are listed under Fatty Acyls, within the main classes, octadecanoids (C_{18}), eicosanoids (C_{20}), and docosanoids (C_{22}).

As more oxylipins continue to be discovered and characterized and interest in their bioactivity and pre-clinical measurement increases, it has become essential for researchers to have access to robust analytical methods that enable their sensitive and selective quantification. It is also important that these methods account for the complexity of oxylipin analysis, while leveraging the high capability of newer-generation liquid chromatography (LC)–tandem mass spectrometry (MS/MS). Over the past 20 years, oxylipin analysis has substantially advanced. Indeed, today's state-of-the-art, targeted LC-MS/MS assays can routinely quantify more than 100 individual molecular species in small amounts of biofluids or tissue extracts, down to low- or sub-picogram amounts on-column in a single analytical run (see Supplementary Materials). Although this is already transforming research into these lipids, there remains a major need to support researchers new to this field who wish to establish these assays. Oxylipins present unique analytical challenges, including low abundance, rapid metabolism to conjugated or chain-shortened forms, the presence of many closely eluting isomers, and similar fragmentation patterns, especially when generated non-enzymatically. Considering this, quantitative analysis of oxylipin families is technically specialist, requiring both chromatography and MS/MS, as well as the availability of authentic and stable isotope–labelled synthetic analytical standards.

The quantification of oxylipins requires accuracy and precision, as well as correct identification and reporting. Given that some oxylipins are present at extremely low endogenous concentrations, it is important to ensure that their measurement adheres to best practices. More broadly, in the wider field of lipidomics, in response to challenges with data reporting and reproducibility, guidelines have been developed, including a Minimal Reporting Checklist (*6, 7*). (More information on these specific issues, with references, is provided in the Supplementary Materials). Following from that work, but specifically supporting researchers interested in performing oxylipin analysis, community recommendations have been developed and are presented here. These summarize the key aspects to be considered when establishing and routinely running a targeted LC-MS/MS method for oxylipin quantitation in research settings and describe those parameters that should be reported in publications. Criteria for routine quantitation are proposed, together with parameters to be reported when establishing new methods. Additional methods, such as high-resolution accurate mass analysis, data-dependent and data-independent fragmentation, ion mobility, and MS imaging are not covered, but in general, the same overall criteria for performance described herein should apply. Our recommendations also contain an extended and fully referenced introduction, providing a comprehensive history of the discovery of oxylipins and their MS/MS analysis.

For targeted analysis in a clinical setting, numerous guidelines for bioanalytical methods already exist, for example from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), the Food and Drug Administration (FDA), and the Clinical & Laboratory Standards Institute (CLSI) (*8-10*). These describe requirements for laboratory methods used in patient care, clinical trials, and diagnostics. However, they do not appropriately address the needs and limitations typically observed in basic research settings aimed at increasing our understanding of the underlying mechanisms of diseases and biological processes. Examples of these limitations include the restricted

availability of standards and reference materials, diversity in sample matrix type and sample origin, as well as the lack of analyte-free matrices. Furthermore, they do not provide specific details related to oxylipins. Addressing this, the new recommendations provide technical advice for oxylipin analysis in laboratories reflecting current state-of-the-art practices in discovery research. Where analysts use oxylipin assays to make measurements for clinical or diagnostic purposes, then the guidelines mentioned earlier also need to be applied.

These community recommendations for laboratory assays for oxylipins were initiated by a working group initially established as an International Lipidomics Society (ILS) Interest Group (https://lipidomicssociety.org/interest_groups/oxylipin-analysis/). After an open advertisement to the biomedical community inviting interested researchers to attend, two webinars were held to discuss basic analytical principles that should be included (87 attendees). After feedback through an online form and by email, a draft was generated and then circulated to the webinar attendees and others for input. After revision, an agreed-upon version was finalized and is presented in the Supplementary Materials. These recommendations are fully aligned with the ILS Minimal Reporting Checklist, which should also be used for data reporting (*6*).

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