Analysis of Complex Mixtures of Octadecanoid Isomers by LC–MS/MS

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The synthesis of twelve oxylipins derived from α-linolenic acid (ALA) and γ-linolenic acid (GLA) facilitates the establishment of an LC–MS/MS method for the detection and quantitation of epoxyoctadecadienoic acids (EpODEs) and dihydroxyoctadecadienoic acids (DiHODEs) in rat plasma.

Introduction

Oxylipins, oxidized metabolites of polyunsaturated fatty acids, are lipid mediators involved in many biological mechanisms of health and disease (1). The best studied are eicosanoids derived from arachidonic or eicosapentaenoic acids, including prostaglandins, leukotrienes, thromboxanes, epoxyeicosatrienoic acids, hydroxyeicosatetraenoic acids, and E-resolvins.

More recently, octadecanoids, oxylipins derived from linoleic or linolenic acids, are being increasingly recognized as having important effects in biological responses (2). However, the availability of authentic standards for these mediators has been limited, making it difficult to analyze complex matrices containing several isomers.

In this study, we used twelve new standards for EpODEs and DiHODEs to optimize LC and MS conditions to analyze octadecanoids in rat plasma.

Experimental Conditions

Twelve octadecanoid standards were synthesized, partially purified, and used to evaluate their chromatographic and mass fragmentation properties to develop a multiple-reaction monitoring (MRM) LC–MS method. Chosen precursor-product ion transitions and retention times are shown in Table I.

TABLE I: MRM transitions and retention times of standards

Octadecanoid	Precursor > Product Ion (*m/z*)	Retention Time (min)
α-9,10-EpODE	293.2 > 171.1	7.25
α-12,13-EpODE	293.2 > 195.1	7.35
α-15,16-EpODE	293.2 > 235.2	7.26
γ-6,7-EpODE	293.2 > 129.1	7.63
γ-9,10-EpODE	293.2 > 169.1	7.60
γ-12,13-EpODE	293.2 > 193.1	7.40
α-9,10-DiHODE	311.2 > 201.1	5.39
α-12,13-DiHODE	311.2 > 241.1	5.47
α-15,16-DiHODE	311.2 > 235.1	5.40
γ-6,7-DiHODE	311.2 > 159.1	5.51
γ-9,10-DiHODE	311.2 > 141.1	6.08
γ-12,13-DiHODE	311.2 > 181.1	5.63

Oxylipins were extracted from rat plasma (BioChemed) using protein precipitation followed by solid-phase extraction (SPE). Extracts were analyzed by reversed-phase LC–MS/MS using an Exion UPLC system coupled with a triple-quadrupole 6500+ mass spectrometer (SCIEX). Analytes were separated on a Kinetex C18 column (100 × 2.1 mm, 1.7 µm, 100 Å) and a gradient of water (1 mM ammonium acetate, 0.01% acetic acid) and 3:1 (v/v) acetonitrile–methanol.

Results

Ten different octadecanoids were detected in rat plasma, as shown in Figure 1. Also shown are examples of MS/MS spectra from two of the authentic standards used during method development. Those spectra were used to choose non-overlapping MRM transitions that allow the independent integration of each signal even when chromatographic resolution of isomers is not achieved.

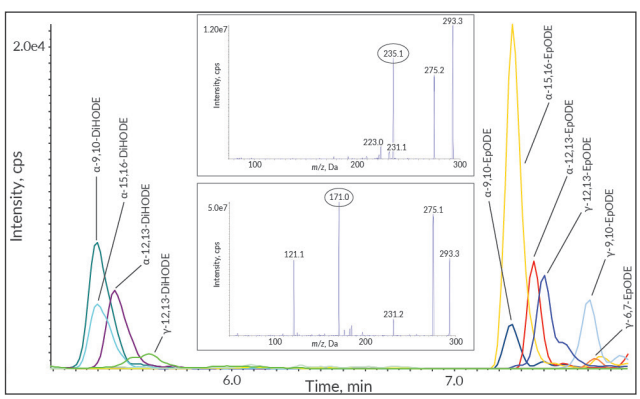

FIGURE 1: LC–MS chromatogram of octadecanoids in rat plasma. Insets: MS/MS spectra of two of the authentic standards used.

Conclusions

The use of authentic standards of several isomeric octadecanoids greatly facilitates the accurate analysis and quantitation of these molecules in complex biological samples. These standards will help investigate the roles of octadecanoids in patients and in experimental models of disease. ■

References

(1) K. Parchem et al. *Prog. Lipid Res.* **95**, 101276 (2024).

(2) J. Revol-Cavalier et al. *Chem. Rev.* **125**, 1–90 (2025).

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