# Improving Selectivity and Sensitivity of Lipid Mediator Analyses by Coupling Nano-flow Chromatography with the Stellar Mass Spectrometer

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## Abstract

Signaling lipids play a central role in biology, mediating a wide range of cellular processes. Over the last few decades, the role of lipids as mediators of inflammation has become increasingly recognized. This process involves biochemical pathways that help inflamed tissues return to homeostasis. Detecting and quantifying these lipids in biological matrices is challenging due to their chemical instability, structural similarity, and low concentrations (typically from ppt to ppb).

Lipid mediator analysis is typically performed by LC-MS/MS using SRM/MRM methods with triple quadrupole mass spectrometers and high-flow UHPLC systems. Although very sensitive, these analyses require optimization of SRM transitions for each individual lipid, making the process cumbersome. To address these challenges, a sensitive targeted approach utilizing a Thermo Scientific™ Vanquish Neo™ UHPLC system operated in the nano-flow regime (500 nL/min) coupled with a Thermo Scientific™ Stellar™ mass spectrometer equipped with a sensitive dual pressure linear ion trap detector is presented.

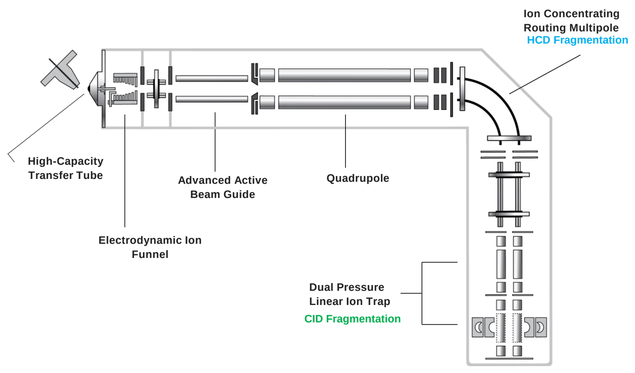
The key benefit of this approach is that the Stellar mass spectrometer uses parallel reaction monitoring (PRM) to monitor multiple transitions simultaneously and can perform both HCD and CID fragmentation with fast acquisition speeds (up to 140 Hz). This method allowed reliable detection and quantification of dozens of lipid species in less than 30 minutes, using less than 20 µL of solvent per sample.

A comparative analysis of the same panel of lipid mediators using a high-flow chromatography method on both the Stellar and Thermo Scientific™ TSQ Altis Plus™ triple quadrupole mass spectrometer was conducted. The nano-flow method demonstrated higher sensitivity than the analogous high-flow method (0.3 mL/min), showcasing another benefit of this approach. Overall, the nano-flow Stellar method is remarkably selective and sensitive, with limits of quantitation (LOQ) reaching low ppt levels.


## Introduction

Here we introduce an optimized workflow to detect lipid mediators using the Stellar mass spectrometer. This instrument is equipped with ion routing multipole for HCD fragmentation and an ion trap for CID and MSn fragmentation, enabling enhanced selectivity (Figure 1). The use of parallel reaction monitoring (PRM) over selected reaction monitoring (SRM) allows simultaneous monitoring of multiple transitions, improving detection efficiency and accuracy.

**Figure 1. Stellar mass spectrometer**




## Materials and Methods

**Sample Preparation:** Lipid mediator standards were obtained from Cayman Chemical and stored at -80 ºC. Calibration curves ranged from 0.0001 to 100 ppb (PUFAs: 0.02 ppb to 20 ppm). Internal standards were spiked at 1 ppb, except for α-linolenic acid-$d_{14}$, which was spiked at 100 ppb.

**Chromatography and Mass Spectrometry:** The nanoflow method used a Vanquish Neo UHPLC at 500 nL/min with the Stellar mass spectrometer. Solvents were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). PRM was employed to monitor multiple transitions with HCD and CID fragmentation. The high-flow method used a Thermo Scientific™ Vanquish Horizon™ UHPLC at 300 µL/min with both TSQ Altis Plus and Stellar mass spectrometers, utilizing the same solvents.

**Analysis:** Data was processed using Thermo Scientific™ TraceFinder™ software for quantitation and identification of lipid mediators. Calibration curves and internal standards ensured accurate quantification. Comparative analysis was conducted between the nano-flow and high-flow methods.

**Figure 2. Workflow schematic**


**Figure 3. Nano-flow and high-flow chromatography methods**

**Vanquish Neo (Nano-flow)**

Thermo Scientific™ EASY-Spray™ PepMap™ Neo Column (0.075 mm x 150 mm, 2 µm)

| Time (min ) | %B |
|---|---|
| 0 | 10 |
| 0.1 | 45 |
| 2.1 | 45 |
| 16.5 | 80 |
| 16.6 | 98 |
| 21.5 | 98 |

Solvent A: Water + 0.1% Formic Acid

Solvent B: Acetonitrile + 0.1% Formic Acid

**Vanquish Horizon (High-flow)**

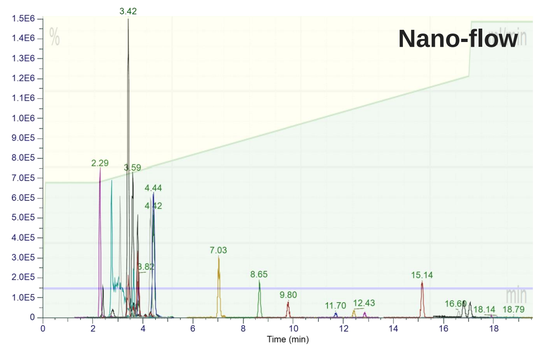
Thermo Scientific™ Hypersil Gold™ Column (2.1 mm x 150 mm, 1.9 µm)

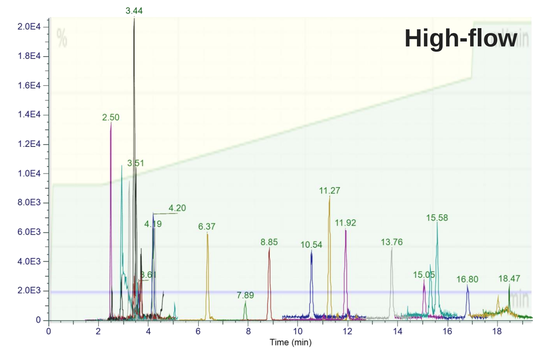
| Time (min ) | %B |
|---|---|
| 0 | 10 |
| 0.1 | 45 |
| 2 | 45 |
| 16.5 | 80 |
| 16.6 | 98 |
| 21.5 | 98 |



Solvent A: Water + 0.1% Formic Acid

Solvent B: Acetonitrile + 0.1% Formic Acid




| Compound Name | Stellar MS Nano-flow LOD/LOQ (ppb) | Stellar MS High-flow LOD/LOQ (ppb) | TSQ MS High-flow LOD/LOQ (ppb) |
|---|---|---|---|
| 6-keto prostaglandin F1α | 0.01/0.01 | 0.5/1 | 0.5/1 |
| Resolvin E1 | 0.05/0.1 | 1/10 | 1/10 |
| Thromboxane B2 | 0.05/0.1 | 1/10 | 1/10 |
| Prostaglandin F2α | 0.01/0.05 | 1/10 | 0.5/1 |
| Prostaglandin E2 | 0.001/0.05 | 0.5/1 | 0.5/1 |
| Resolvin D3 | 0.01/0.1 | 1/1 | 1/1 |
| Lipoxin B4 | 0.05/0.5 | 10/10 | 10/10 |
| Prostaglandin D2 | 0.05/0.1 | 10/10 | 10/10 |
| Resolvin D2 | 0.05/0.5 | 10/10 | 10/10 |
| Lipoxin A4 | 0.001/0.05 | 1/10 | 1/10 |
| 17(R)-Resolvin D1 | 0.01/0.05 | 10/10 | 10/10 |
| Resolvin D5 | 0.01/0.05 | 0.1/0.5 | 0.1/0.5 |
| 12(S)-HHTrE | 1/1 | 0.5/1 | 10/10 |
| (+)18-HEPE | 1-10 | 0.5/1 | 0.5/1 |
| 15(S)-HETE | 0.01/0.5 | 10/10 | 10/10 |
| 12(S)-HETE | 0.05/0.1 | 0.5/1 | 1/10 |
| 5(S)-HETE | 0.01/0.05 | 10/10 | 10/10 |
| Stearidonic Acid | 10/20 | 200/2000 | 2000/2000 |
| Eicosapentanoic Acid | 200/200 | 200/200 | 100/200 |
| α-linolenic acid | 200/200 | 2000/2000 | 2000/2000 |
| γ-linolenic acid | 200/200 | 200/2000 | 2000/2000 |
| Docosahexanoic acid | 2000/2000 | 200/2000 | 100/200 |
| Arachidonic Acid | 2000/2000 | 200/2000 | 200/2000 |

**Table 1. Comparison of LOQ's and LOD's of lipid mediators using nano-flow and high-flow chromatography with the Stellar MS and high-flow chromatography with the TSQ Altis Plus MS**

| Feature | Stellar | TSQ Altis Plus |
|---|---|---|
| Dissociation | In-source CID, HCD (Collison cell), CID (resonance) | In-source CID, Collision cell CID |
| MSn Capability | Yes | No |
| Sensitivity | +++++ | +++++ |
| Resolution | Low (nominal mass) | Low (nominal mass) |
| Scan Speed | 140 Hz | 600 SRM/s |
| Monitoring Mode | PRM | SRM |
| Transition Monitoring | Simultaneously | Sequential |
| Mass Range | m/z 5-2000 (full scan) m/z 30-2000 (MS2, MS3) | m/z 5-2000 |
| Polarity Switching | 5 ms | 5 ms |
| Tools for quantitation | Retention time alignment | Compound Optimization |

**Table 2. Comparison of the Stellar and TSQ Altis Plus mass spectrometers for targeted quantitation**

## Results

**Figure 4. Calibration curves for Prostaglandin E2 using (A) nano-flow with the Stellar (B) high-flow with the Stellar MS and (C) high-flow with the TSQ Altis Plus MS**

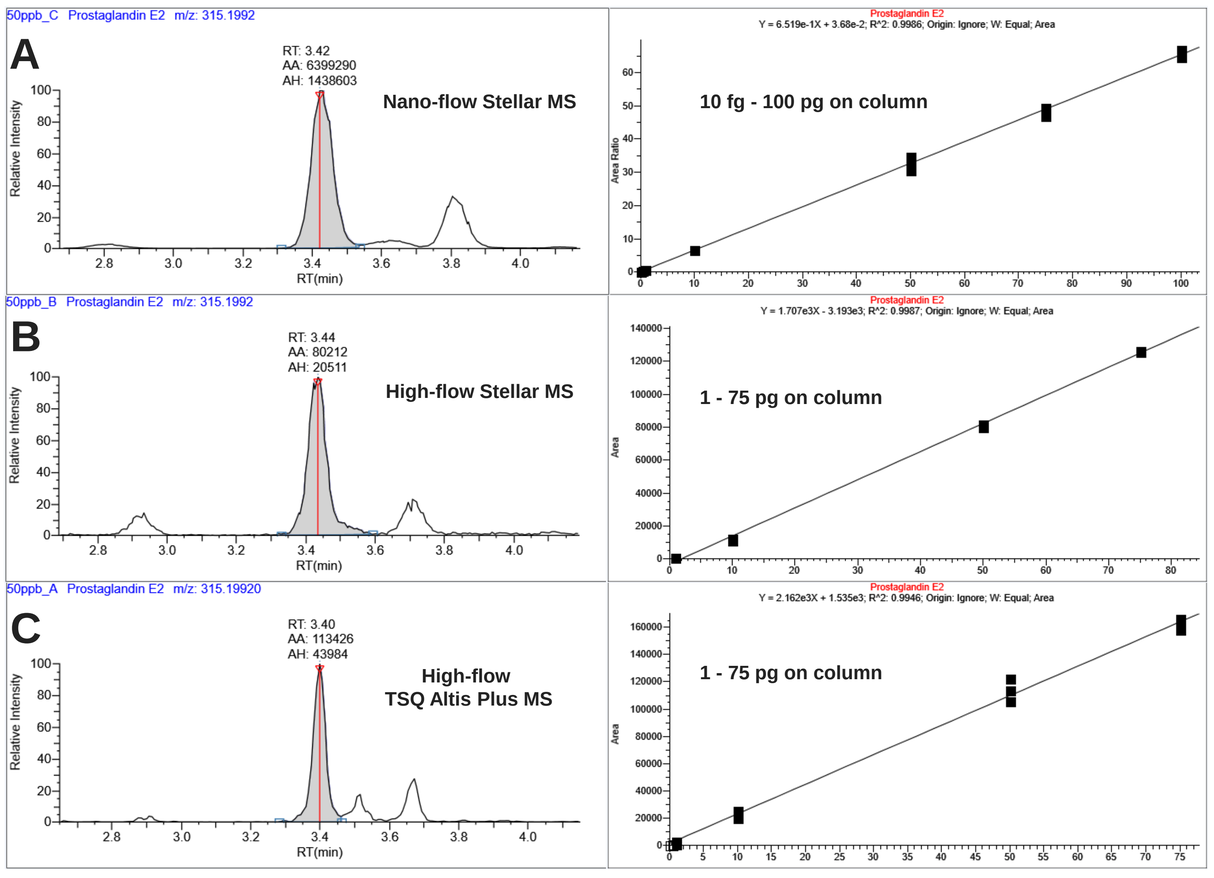
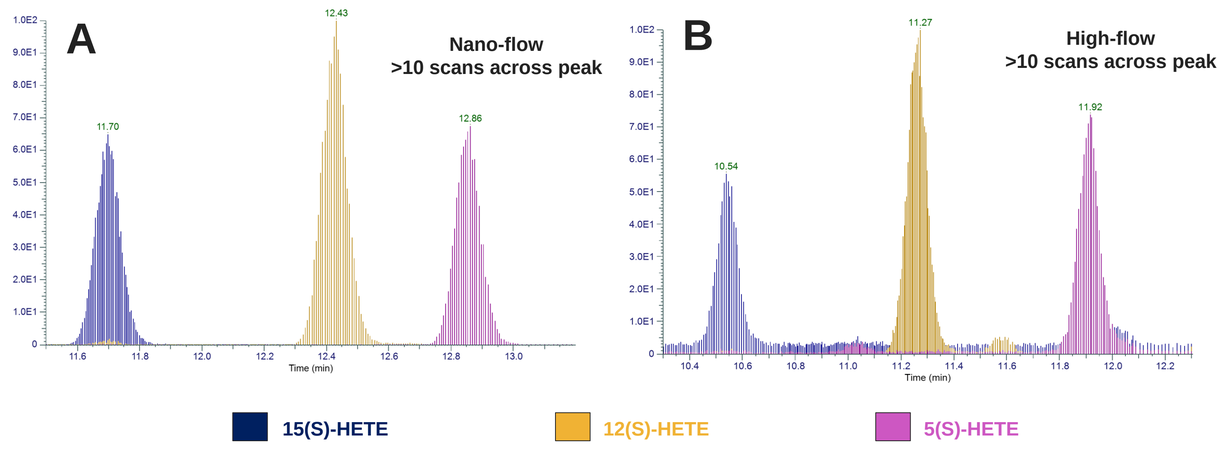



**Figure 5. Extracted ion chromatograms of 15(s)-HETE, 12(S)-HETE and 5(S)-HETE using (A) high-flow and (B) nano-flow with the Stellar mass spectrometer**




## Conclusions

- The nano-flow chromatography coupled with the Stellar mass spectrometer significantly enhances the sensitivity and selectivity of lipid mediator analyses.
- This advanced method enables reliable detection and quantitation of lipid mediators at extremely low ppt levels, surpassing the capabilities of traditional high-flow methods.
- The parallel reaction monitoring (PRM) feature of the Stellar mass spectrometer allows for the simultaneous monitoring of multiple transitions, improving detection efficiency.
- The workflow is more efficient and cost-effective, requiring less solvent and sample volume.

## Trademarks/licensing