TARGETED SPE-UPLC-MS/MS ANALYSIS OF OXYLIPINS: FROM PROFILING TO QUANTIFICATION FOR TRANSLATIONAL RESEARCH STUDIES

Billy Joe Molloy
Waters Corporation, Wilmslow, UK

INTRODUCTION

Oxylipins are signaling molecules that play a role in the regulation of many key biological processes, most notably inflammation. Here, we describe the development of targeted, quantitative solid phase extraction - ultra performance liquid chromatography - tandem mass spectrometry (SPE-UPLC-MS/MS) assays for the analysis of two subsets of oxylipins. These subsets represent down-stream products, or oxylipins, from particular precursor lipids and metabolic pathways. Developing separate analytical methods for subsets of analytes is a more specific approach, giving superior results overall when compared to profiling methods, which tend to be more generic, compromising on performance. Matrix samples were prepared using mixed mode Oasis MAX µElution SPE and analysed using an ACQUITY UPLC 1-Class system interfaced to a Xevo TQS Micro tandem quadrupole mass spectrometer. We demonstrate these methods to be sensitive, selective, linear and precise and therefore suitable for use in quantitative translational research studies.

METHODS

Sample preparation/SPE
All standards were from Cayman Chemicals and used to prepare sets of combined solvent calibrators (40 - 20,000 pg/mL) for each subset. Human serum was from Golden West Biologicals, DMEM buffer and FBS were from Sigma-Aldrich and CD-1 mouse livers were from Biochemed Services. All samples were diluted and then prepared using Oasis MAX mixed mode µElution SPE. Briefly, samples were loaded onto a conditioned plate, washed, and the analytes eluted with an IPA/ACN/formic Acid mixture into a collection plate containing dilution solution. Samples were mixed and 10 µL injected/analysed.

LC-MS/MS
UPLC-MS-MS analysis was performed using an ACQUITY UPLC 1-Class System. Analytes were injected onto a 2.1 x 150 mm BEH C18 column using a water/acetonitrile/formic acid gradient and detection was achieved using a Xevo TQ-S Micro Detector operated in negative ESI mode. Precision was determined by extracting and quantifying samples at various concentration levels (n=6) in various matrices against the solvent calibrators. The specificity of the chromatographic method employed was tested by running potential isobaric interferences as solvent standards in order to determine if they co-eluted with the analytes of interest. Recovery was assessed by comparing pre-extraction spiked matrix against post-extraction spiked matrix. Matrix effects were assessed by comparing the relative response between the internal standard in the solvent standards and the relevant matrix samples.

RESULTS

Separate mixed mode SPE methods were developed for two subsets of oxylipins, containing products of the cyclooxygenase (COX) and lipoxygenase (LOX) metabolic pathways. The COX subset contained prostaglandins D2, E2 and F2a (PGD2, PGE2 and PGF2a), 6-keto-prostaglandin F1α (6-Keto-PGF1α) and Thromboxane B2 (TBX2). The LOX subset 5-, 8-, 12-, and 15-positional isomers of hydroxyeicosatetraenoic acid (5-HETE, 8-HETE, 12-HETE and 15-HETE), and the 9- and 13- positional isomers of hydroxyoctadecadienoic acid (9-HODE and 13-HODE). The recovery of all analytes was shown to be above 75% (Figure 4), and matrix effects were negligible. This combination of high recovery and low matrix effects resulted in both methods being highly sensitive (LOD = 10 - 100 pg/mL) in multiple matrices.

Separate chromatographic methods were developed for the two oxylipins subsets. All analytes were separated from each other and other isomeric/isobaric species. For example, PGF2a was shown to be baseline resolved from 5-Trans- PGF2α, 8-isopGF2a, 15(R)-PGF2a, 8-iso-15(R)-PGF2a, PGF2β, 5-Trans-PGF2β and 8-iso-PGF2β. Using the same method, PDG2 and PGE2 were also shown to be baseline resolved from 15(R)-PGE2, 8-iso-PGE2 and 11β-PGE2. (Figures 1 and 2). Figure 2 shows that in FBS matrix an unknown isobaric species is interfering with the PGD2 peak at low levels. Both methods were reproducible (<15%CV) in multiple matrices and linear to 20 ng/mL for all analytes.

CONCLUSION

- Separate SPE-UPLC-MS/MS methods have been developed for two subsets of oxylipins. The importance of specificity has been demonstrated for the quantitative analysis of these analytes from biological matrices. In particular, the chromatographic separation of isomeric forms of the analytes.
- The benefits of having specific methods for specific subsets of analytes, as opposed to a generic profiling method, have been demonstrated. These methodologies are ideally suited to quantitative translational research studies.

FOR CLINICAL RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS

©2016 Waters Corporation