

Introduction:

- Phosphatidylethanol (PEth) is a direct, specific alcohol biomarker for monitoring alcohol consumption over time
- Accurate identification and quantification of PEth is analytically demanding and challenging due to the reference standards availability

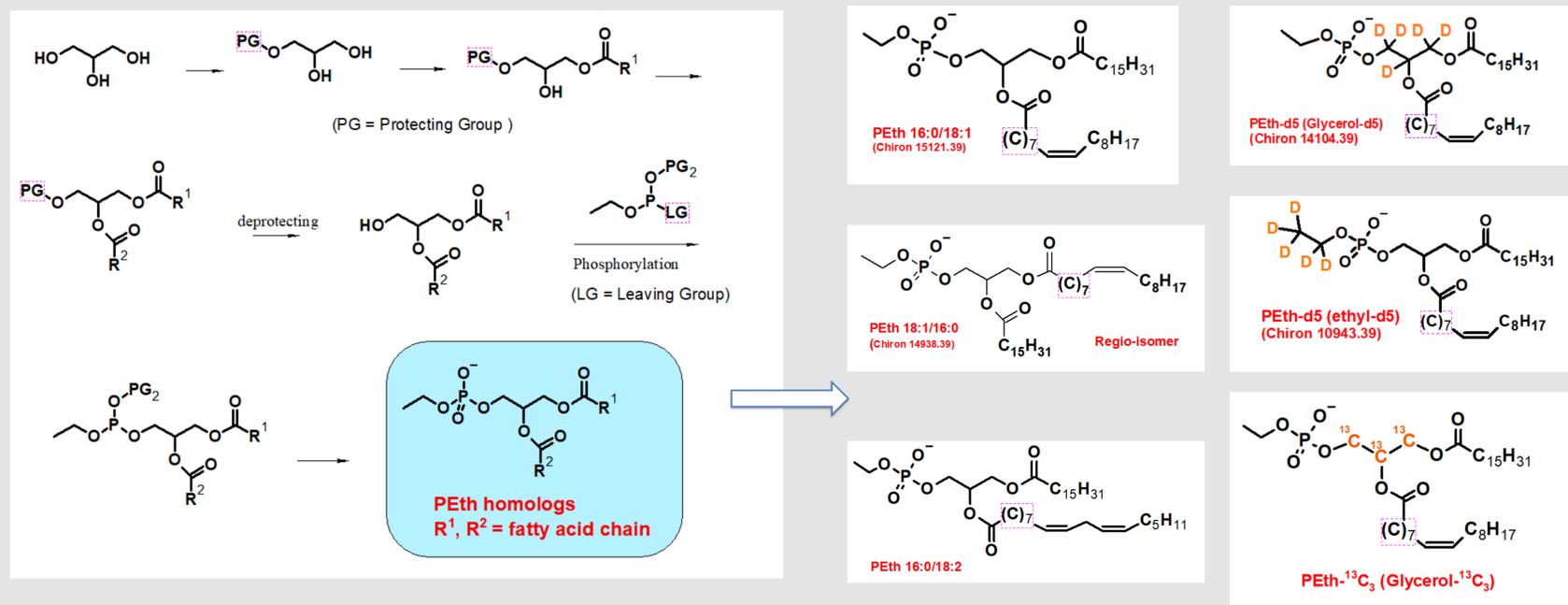
Objectives:

- Develop novel chemical synthesis methods to increase the production volumes of PEth reference materials and deuterated, ¹³C-labelled PEth 2.
- Improve the quality/purity of PEth reference materials; Address regio-isomers impact on toxicological analysis

Materials and Methods:

1. Chemical synthesis of PEth and labelled PEth standards

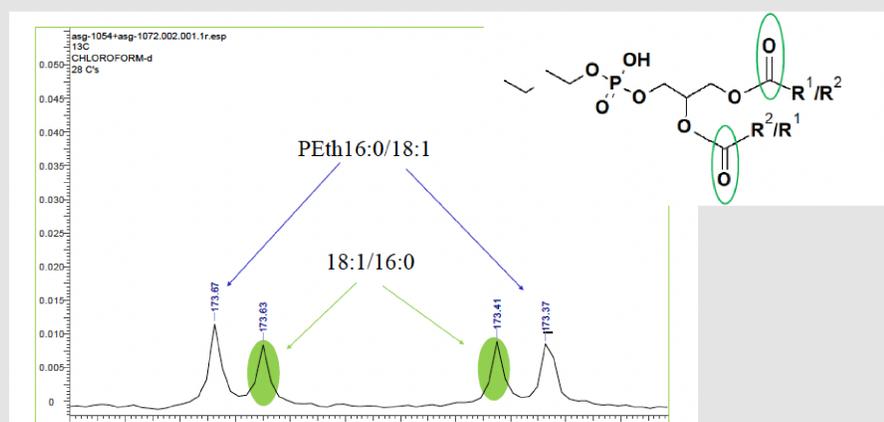
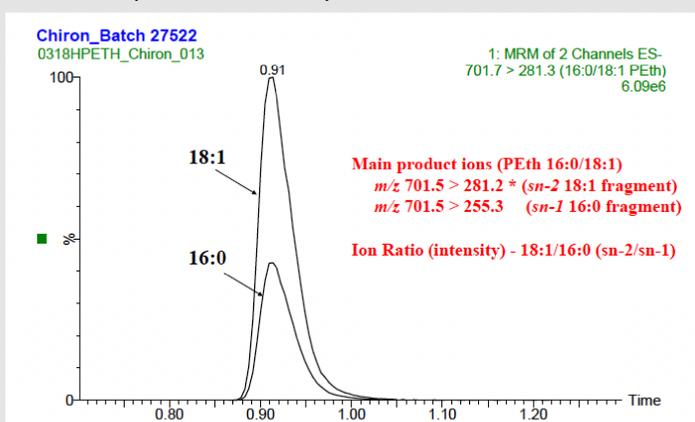
Chemical synthetic methods were developed for the synthesis of individual PEth homologues and stable isotope labelled PEth Internal standards. Levulinyl protection in one of the crucial steps were chosen especially for PEth having one or more double bonds in the fatty acid at sn-2 position, and for PEth internal standards having deuterium or ¹³C-isotopic labelling in the glycerol moiety



2. PEth regio-isomer purity and ¹³C-NMR analysis

Regio isomers, same mass, identical on LC-MS --- Deviation on Ion ratio for quantification by LC-MS/MS

¹³C-NMR of Carbonyl - mixture of PEth 16:0/18:1 and 18:1/16:0



Results and Discussion

- 1) PEth (16:0/18:1), PEth(16:0/18:1)-d5 (ethyl-d5) and PEth (16:0/18:1)-d5 (glycerol-d5) and ¹³C-labelled PEth (16:0/18:1)- ¹³C₃ (glycerol¹³C₃) have been synthesized
- 2) Compared to enzymatic route, chemical synthesis of PEth may provide higher quality and more reproducible reference standards.
- 3) The synthesized PEth reference materials were obtained in high chemical purity and regio-isomeric purity.
- 4) Reversed position isomer PEth (18:1/16:0) was also synthesized for comparison and identification of regio-isomers and regio-isomeric purity.
- 5) Differentiation of the regio-isomers by 600MHz and 800MHz NMR was performed and different ¹³C-NMR signals of the carbonyl groups were observed and identified.
- 6) PEth reference materials are available as commercial products from Chiron AS.

Conclusion

- 1) Chemical synthetic pathways have been developed for the synthesis of PEth homologues.
- 2) With the synthetic methods developed it is possible to prepare highly pure PEth homologues without unwanted regio-isomers and without other chemical impurities
- 3) Isotopically labelled PEth with deuterium and ¹³C-labelled glycerol moieties have also been prepared as internal standards for quantitative analysis of PEth.

Patent application EP3971192A1 - «Phosphatidylalkanol homologues having labelled moieties», 2019, Chiron AS
United States Patent US 9347961- « Test kit for Quantitative Determination of Narcotic Drugs», 2015, Chiron AS