PRAHEALTHSCIENCES

EBF-Open Symposium "go see the doctor"

Patient: Method for the determination of Resolvin E1 in human plasma

DATE
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PRESENTED BY
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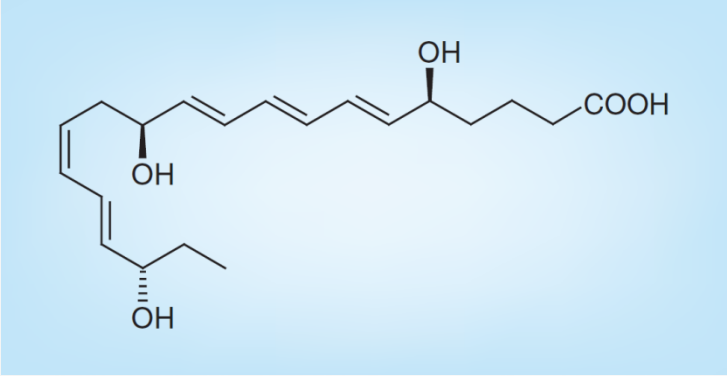
Background

Omega-3 fatty acids are known for there benefit against inflammatory disorders.

This is largely due to the endogenous conversion of omega-3 fatty acid eicoapentaenoic acid (EPA) into mediators called Resolvins:



Resolvin E1

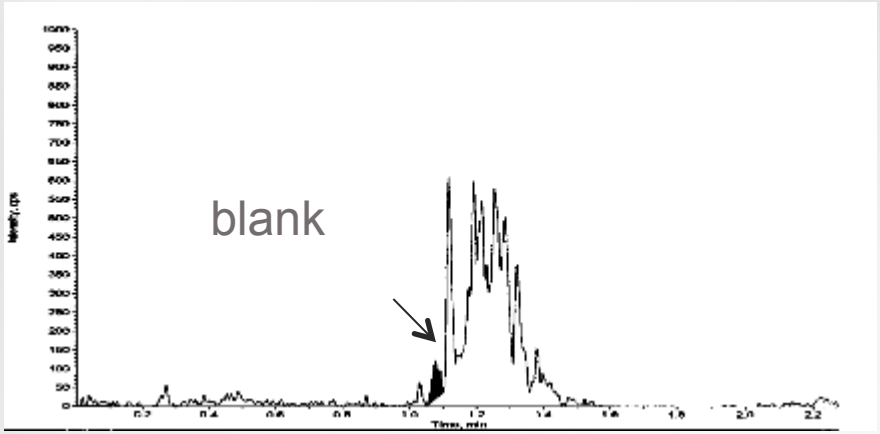
A bioanalytical method was developed to analyze Resolvin E1 to support a first in man clinical trial

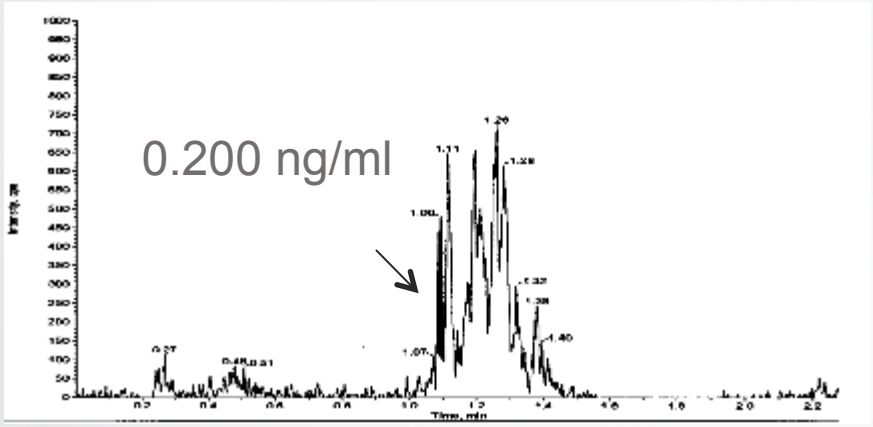
Method overview

- 100 µl plasma
- 20 µl IS (structural analogue)
- 500 µl 0.1% formic acid
- Extracted with 3 ml acetonitrile: n-chlorobutane 3:7 v/v
- Evaporation
- Redissolved in 100 µl Methanol: water (3:7 v/v)
- 10 µl injection

Gradient:	from 25 to 50% ACN in 0.05% aqueous formic acid in 1.2 min followed by 95% ACN till 1.5 min and re-equilibration at 25% until 2 min
Column:	UPLC C18, 1.7 µm particles
Detection:	negative mode ESI-MSMS m/z 349.1 - 107

Symptoms





Almost all blank plasma lots showed interfering peaks at the retention time of Resolvin E1

Due to the interferences, an LLOQ of 5 ng/ml is the best achievable whereas 0.200 ng/ml is needed.

Ideas?

Attempts to improve selectivity

Gradient changes	No success
Other analytical columns	No success
Change of sample preparation	No success
UPLC	No success
Other mass transitions	No success

Interfering peaks are probably strongly structural related (fatty acids?)

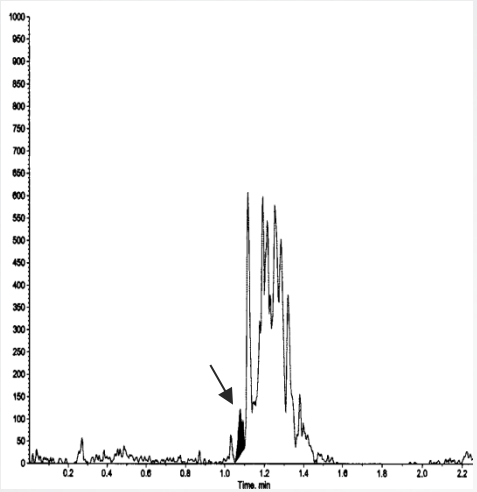
High Variability between plasma lots

Diet dependent? Problematic in the clinic!

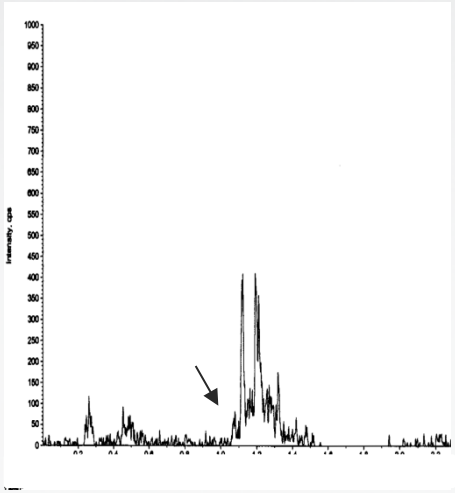
Ideas?

Observation

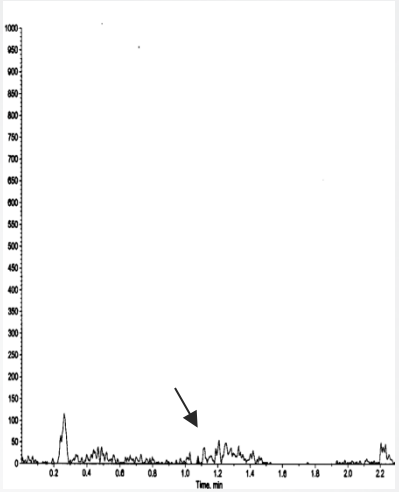
It was observed that the peak abundance was related to plasma age:



238 days



71 days



fresh

Peaks probably originate from oxidation products of fatty acids formed during storage.

Cure?

Analyse samples shortly after collection and use calibrators and QC samples obtained from freshly processed plasma.

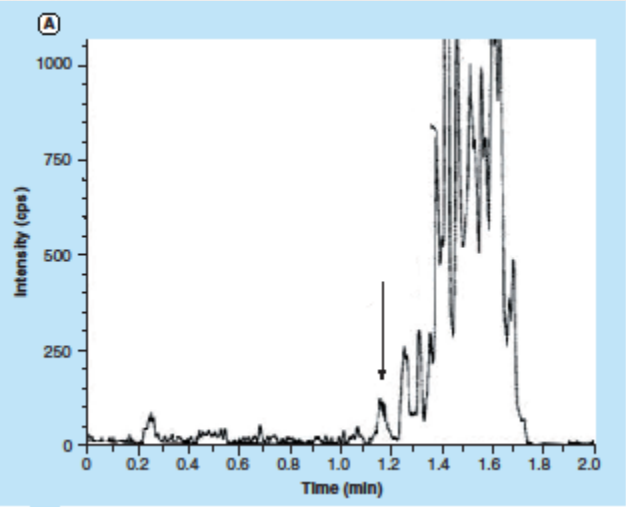
But… During storage interfering peaks will be formed at an unknown rate preventing reliable reanalysis when needed.

Therefore, use of the addition of antioxidants was investigated to prevent the formation of degradation products.

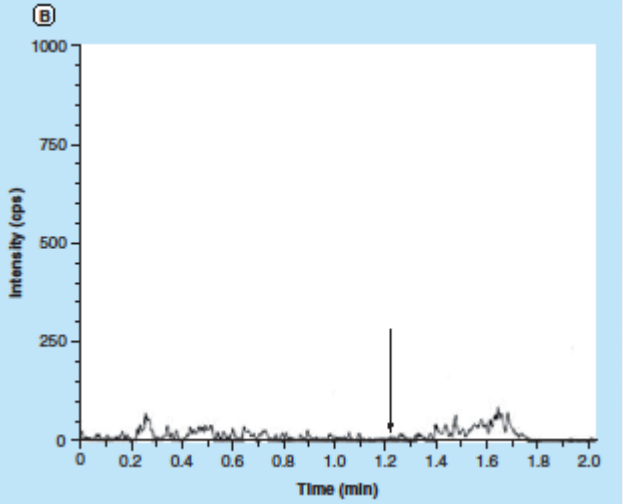
- Bisulphate
- Glutathione
- Ascorbic acid
- butylated hydroxytoluene (BHT)

Cure?

Fresh blank plasma was stressed by storage for 7 days at room temperature



Without BHT



With BHT

Cure!

- Prepare calibrators and QC samples in freshly processed plasma
- Addition of antioxidant BHT immidiately after plasma processing to all samples

Patient cured !

Questions?