



## R-TCH-32

# Targeted Metabolomics for Stability Testing in Autologous Serum Eye Drops after Storage of 9 Months

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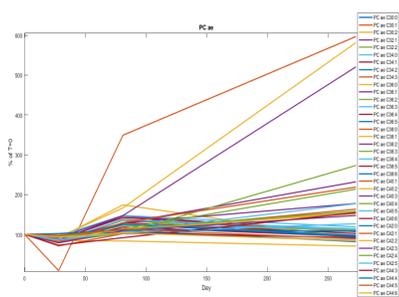
**Background** Autologous serum eye drops (ASED) are often used for the treatment of dry eye syndrome. The use of ASED comes with uncertainties regarding serum storage times longer than 1 month and any dilution factor.

**Objectives** To assess the stability of metabolites in ASED under storage conditions at  $-20^{\circ}\text{C}$  for up to 9 months and potentially reduce the frequency of blood donations from patients and the logistics involved.

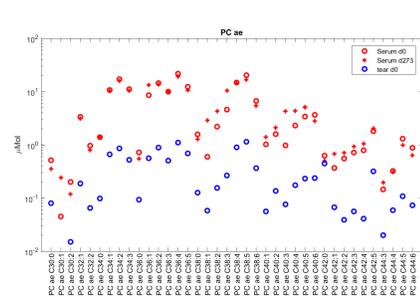
**Methods** 450 ml of autologous whole blood was stored for 2 hours at  $20\text{-}24^{\circ}\text{C}$  and 12 hours at  $2\text{-}6^{\circ}\text{C}$ . Coagulated whole blood was separated by two sequential 15 minute centrifugation cycles ( $6407\times\text{g}$  at  $16^{\circ}\text{C}$  and  $3876\times\text{g}$  at  $4^{\circ}\text{C}$ ) and manual compression. Unit-dose ASED (1.5 ml) were prepared and stored at  $-20^{\circ}\text{C}$  for 9 months. Concentration changes of 76 phosphatidylcholines (PC), 14 sphingomyelins (SM), 14 lysophosphatidylcholines (LPC), 21 amino acids (AA), and the combined hexoses were determined on days 0, 28, 81, and 273 by LC-MS/MS using the Absolute/DQ®-p180-Kit (Biocrates Life Sciences) and compared to those in tears of the same person.

## Results

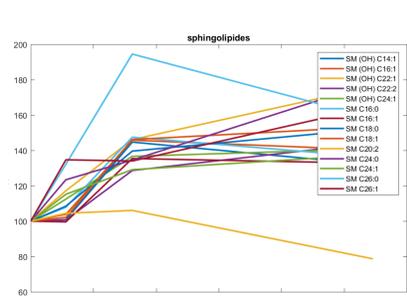
% PCae in serum relative to day 0 over 9 months (storage at  $-20^{\circ}\text{C}$ )



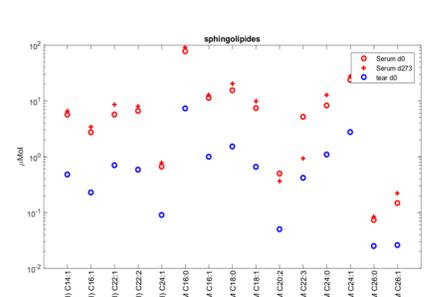
concentrations of PCae in serum at d0, d273 and in the tears



% SM in serum relative to day 0 over 9 months (storage at  $-20^{\circ}\text{C}$ )



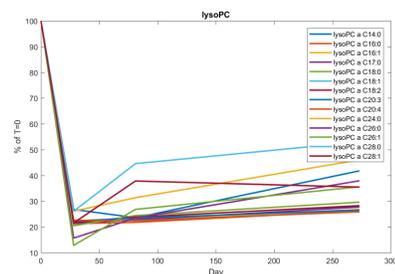
concentrations of SM in serum at d0, d273 and in the tears



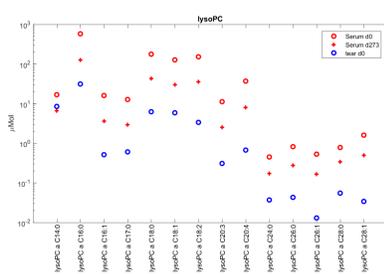
	Serum d0	Serum d273	Tears	Serum (d273) / Tears
Σ PCae	184 µmol/l	224 µmol/l	11.5 µmol/l	19

Most PC showed a less than 2-fold increase, while PC ae C30:1/C38:1/ C38:2 increased by 5-6 fold with most of the increase occurring between days 81 and 273 of storage. "ae" denotes that one of the moieties is a fatty alcohol and bound via an ether bond.

% LysoPC in serum relative to day 0 over 9 months (storage at  $-20^{\circ}\text{C}$ )



concentrations of LysoPC in serum at d0, d273 and in tears



	Serum d0	Serum d273	Tears	Serum (d273) / Tears
Σ LPC	1133 µmol/l	312 µmol/l	57.5 µmol/l	5

The concentrations of all LPC decreased within 28 days by 50%-75%.

## Acknowledgements

Ines Maria Hartmann<sup>1</sup>, Jasmin Leweke<sup>2</sup>

	Serum d0	Serum d273	Tears	Serum (d273) / Tears
Σ SM	165 µmol/l	235 µmol/l	16.9 µmol/l	14

The concentrations of all sphingomyelins increased by 30% to 80% with most of the change occurring within the first 81 days.

Also primarily within the first month, we observed reductions of AA between 10% (phenylalanine) and 80% (methionine, ornithine). By the end of the test period, the level of hexoses only decreased minimally by about 15%. When comparing to tears, we found that the metabolite concentrations in serum, even after long term storage, even those that declined, are a multiple fold higher than in tears.

**Conclusions** We observed a shift from LPC to PC most likely through the presence of LPC-acyltransferases. The increase in SM may be linked to the release of sphingosine-1-phosphate from platelets during blood clotting. After 9 months, the LPC levels exceeded those in tears by at least 5-fold. The finding that the metabolite concentration in serum is a multitude higher than in tears justifies to dilute the serum 5-fold to generate ASED. This data also support a storage time of 9 months at  $-20^{\circ}\text{C}$  and thus a less frequent blood donation schedule is possible.