LIPIDOMIC PROFILING OF DRIED SERUM SPOTS (DSS)

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1 DSS METHOD OPTIMIZATION

Dried Serum Spots (DSS) offer a number of advantages like easy and cheaper sample transportation at room temperature as well as sample conservation and storage, facilitating thus multicenter studies or shipping samples.

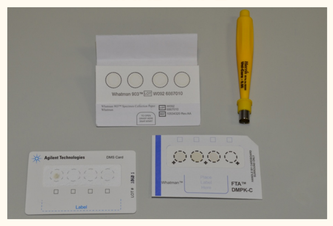
- **CARD SELECTION**
  - CARD TYPE
  - SPOTTED SERUM VOLUME
  - PUNCH SIZE
- **SAMPLE STABILITY**
  - SERUM BEHAVIOUR ALONG TIME
  - REPRODUCIBILITY AMONG DIFFERENT SPOTS
- **DSS VS. LIQUID SERUM**
  - COMPARISON BETWEEN DRIED SERUM SPOTS AND LIQUID SERUM EXTRACTIONS

Method Development Flowchart. Steps followed for the implementation of a lipid method extraction in DSS.

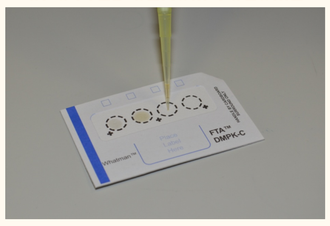
2 CARD SELECTION

Type of cards, punch sizes and extraction procedures were selected based on the amount of recovered compounds and their chromatogram intensities, as well as the variety of extracted lipids. The best results were achieved with Agilent DMS cards and a punch of 3 mm. Cards were loaded with the maximum volume of serum permitted, being 100 µL for Agilent DMS cards.

CARD TYPE



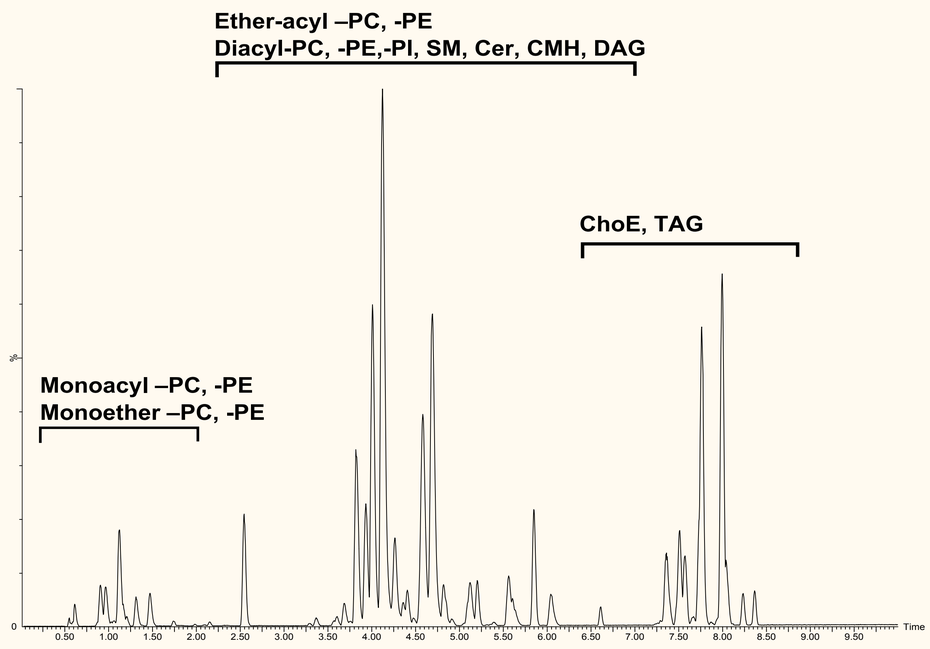
SPOTTED SERUM VOLUME



PUNCH SIZE

The assay consisted of a simple solvent extraction with chloroform/methanol of a disk punched from the center of the DSS card, followed by reverse phase separation using an UPLC-BEH C18 column in combination with a tandem Xevo G2 QTof mass spectrometer (Waters Corp.)[‡].

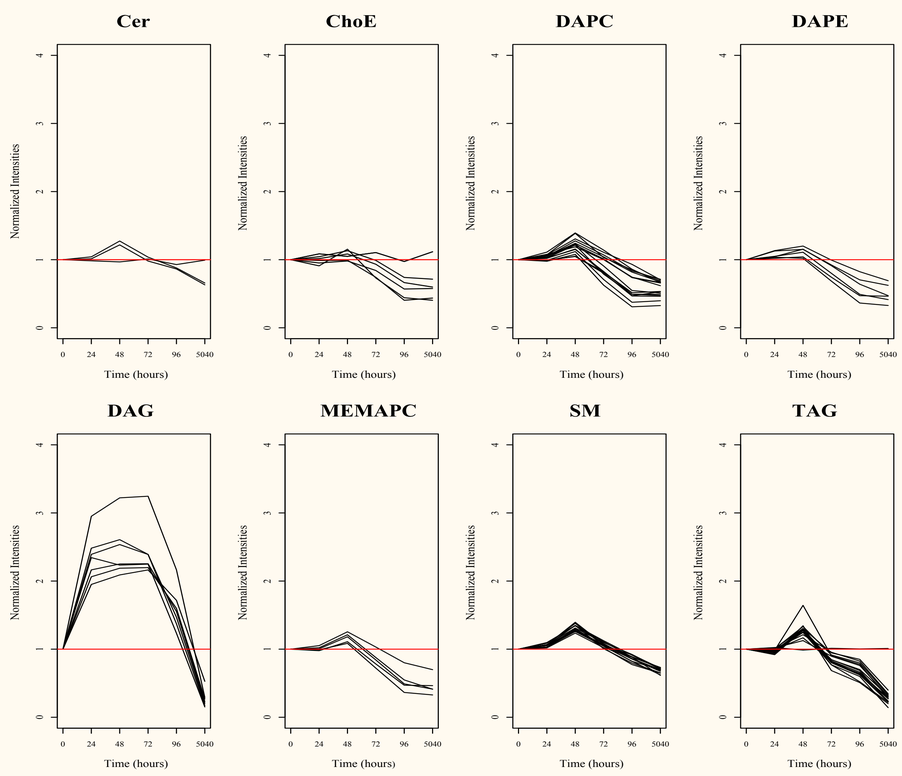
[‡]Barr, J. et al. J. Proteome Res. 2012, 11, 2521-2532.



BPI Chromatogram of a DSS Sample. Among the identified lipids there are ceramides (Cer), sphingolipids (SM), monohexosylceramides (CMH), phosphatidylcholines (DAPC, MEMAPC), phosphatidylethanolamines (DAPE, MEMAPE), phosphatidylinositols (DAPI), diacylglycerides (DAG), triacylglycerides (TAG) and cholesteryl esters (ChoE).

3 SAMPLE STABILITY

Lipid species show significant differences along time, decreasing their intensities with time. Trend curves are different among lipid classes, however, similar behaviour is observed within the same class. Several spots were analyzed at each time point and no significant differences were found among their values.



Interaction Graph. Sample extraction at different times, from 0 to 5040 hours, considering time 0 when samples were already dried (approximately two hours after sera were deposited onto the cards). For most metabolites, intensities increase during the first hours, showing the maximum intensity at 48h. In contrast, DAGs show a different trend and their intensities continue increasing up to 72h.

4 COMPARISON DSS VS. LIQUID SERUM

Correlation Chart: Pearson Test. Up to 76% of studied lipid families present a significant correlation (red dash line) between DSS and liquid serum samples.
Fold-change Chart: The levels of studied lipids are about 60% lower in DSS than in liquid serum, except for those of the DAG family, which values are only 20% lower.

5 CONCLUSIONS

- A simple method to obtain the lipidomic profile in DSS has been optimized.
- Levels of lipid species show significant differences along time though similar behaviour is observed within the same class.
- Lipid levels in DSS are lower than those in liquid serum for all studied metabolites.

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