Steno Diabetes Center Copenhagen
Systems Medicine - Metabolomics Laboratory

Development and comparison of sample preparation methods for LC-MS based lipidomics on fecal samples

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OVERVIEW

- New tools for understanding early metabolic changes in type 2 diabetes needed

  → we developed and compared sample preparation techniques for LC-MS based lipidomics analyses of fecal samples

- A modified Folch-extraction procedure provides reasonable amount of annotated lipid features from fecal samples with acceptable recovery and repeatability

- The developed method ensures faster and more robust analysis, with a broad lipid coverage, suitable for high throughput lipidomics

INTRODUCTION

- The gut microbiota seems to play a role in type 2 diabetes (T2D) – a disease with globally increasing prevalence

  → researchers trying to develop new tools for understanding early stages of T2D and how it is influenced by the host and microbial metabolism

  → lipidomics could provide additional input to diabetes research and help understand the functionality of the gut microbiota

METHODS

- Three different liquid-liquid extraction sample preparation protocols were implemented on fecal slurries (mixture of feces and water) according to Table 1.

- The samples were analyzed on an in-house lipidomics platform (UHPLC-Q-TOF-MS)

- The data was processed using MZmine (version 2.28) and our in-house lipidomics library

Table 1. Overview of performed tests for method development.

Extraction technique	# of test set	Sample amount (~ mg)	# of repetitive extraction steps
EtOH	1	50	1 3
MeOH:MTBE – according to Matayesh *et al.*[1]	1	50	1 3
$CHCl_3$:MeOH - Modified Folch-extraction[2]	1	50	1 3
$CHCl_3$:MeOH	2	10 25 50 75 100	1

REFERENCES

[1] Matyash, V., Liebisch, G., Kurzchalia, T.V., Shevchenko, A., Schwudke, D. *J. Lipid Res.* **49** (2008) 1137-1146.
[2] Folch, J., Lees, M., Stanley, G.H.S. *J.Biol. Chem.* **226** (1957) 497-509.

Table 2. Annotated lipid features in test sets 1 and 2.

Lipid class	# in 1 × EtOH	# in 3 × EtOH	# in 1 × $CHCl_3$:MeOH	# in 3 × $CHCl_3$:MeOH	# in 1 × MeOH:MTBE	# in 3 × MeOH:MTBE	$CHCl_3$:MeOH # in 10 mg	# in 25 mg	# in 50 mg	# in 75 mg	# in 100 mg
DG	2	2	1	1	1	1	1	1	1	1	1
CE	3	3	2	2	2	2	3	3	3	4	4
LPC	12	11	10	11	10	10	8	7	7	6	5
PC	46	23	44	41	40	38	51	63	55	55	52
PE	3	2	1	1	1	1	3	2	2	2	1
SM	1	1	15	14	15	14	11	16	16	16	10
TG	77	79	108	100	105	103	119	121	126	134	124
Total	**144**	**121**	**181**	**170**	**174**	**169**	**196**	**213**	**210**	**218**	**197**

RESULTS

- Using $CHCl_3$:MeOH as the extraction solvent (without repetition) provides a higher number of annotated lipid features as compared to MeOH:MTBE and EtOH (Table 2)

  - changes in peak areas for internal standards correspond to the number of annotated lipid features thus supporting the use of $CHCl_3$:MeOH for future work (Figure 1)

- 25 mg and 50 mg samples show similar numbers of annotated lipid features with no peak saturation (Table 2)

  → 10 mg samples show fewer annotated lipid features per lipid class while 75 mg and 100 mg samples result in saturation of multiple lipid peaks (Table 2)

- Relative standard deviations (%RSDs) of the peak areas for the internal standards (Figure 2) and for 7 randomly selected annotated lipids (Figure 3) were calculated for replicates of the 25 mg and 50 mg samples

  → %RSDs below 10% for the internal standards and below 15% for most of the randomly selected lipids for the 25 mg samples

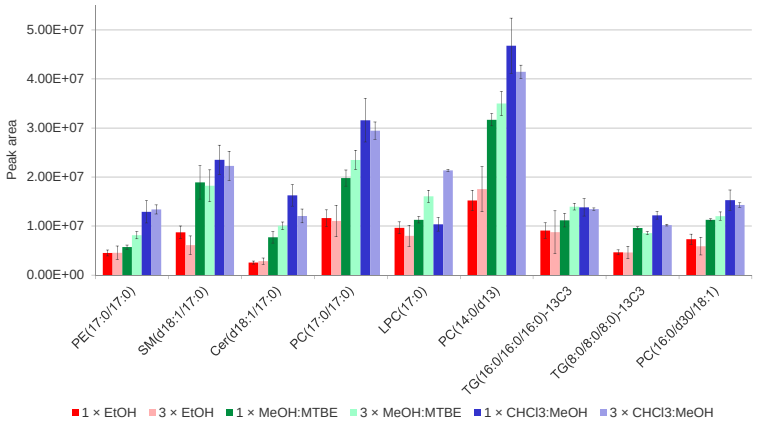

Figure 1. Peak areas of internal standards using different sample preparation protocols.

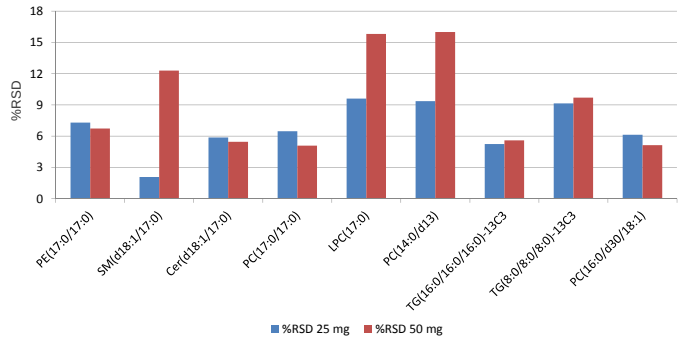

Figure 2. %RSDs of peak areas for internal standards in the 25 mg and 50 mg samples.

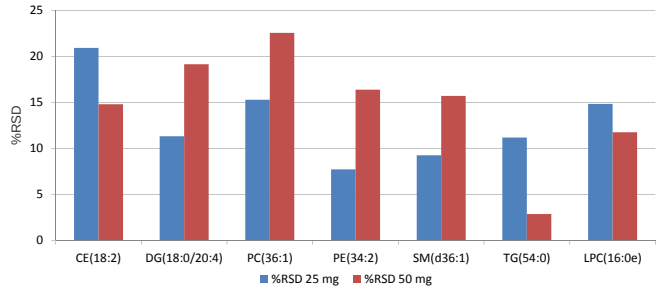

Figure 3. %RSDs of peak areas 7 randomly selected annotated lipids in the 25 mg and 50 mg samples.

CONCLUSIONS

- The modified Folch extraction procedure using 25 mg of fecal slurry demonstrated the best results with respect to recovery and repeatability

- The final performance of the method has been confirmed using a small test set of actual patient samples and the method is implemented in our laboratory to study fecal lipid changes