High-Throughput Preparation of Cellular FAMEs and Sterols for GC/MS Analysis

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Abstract

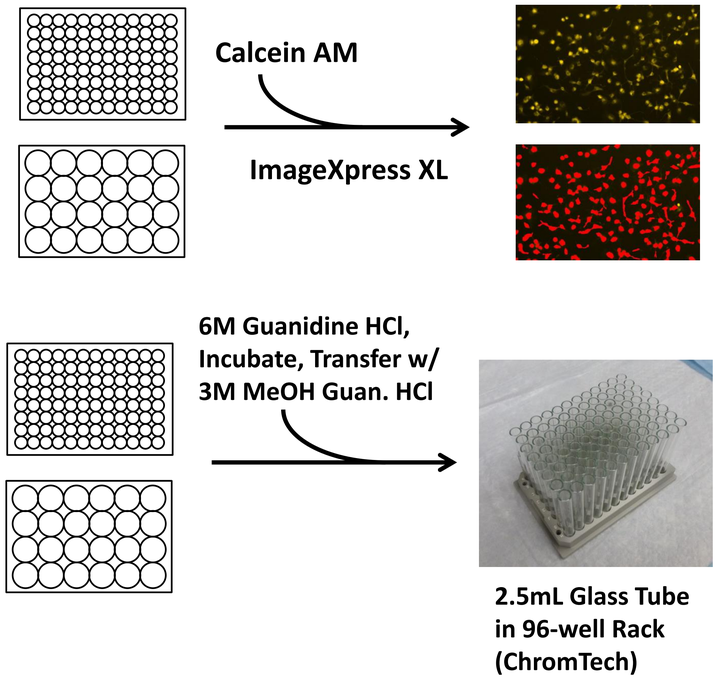
In recent years, metabolomic and isotopic enrichment analysis has led to breakthroughs in a variety of fields of research including cancer biology, immunology, and aging/regenerative medicine. This research has necessitated the preparation and analysis of ever increasing numbers of samples. There are considerable challenges to preparing metabolomics samples, especially in the preparation of cellular lipid samples. Here we describe a high-throughput procedure for the preparation of cellular fatty acids and sterols that reduces costs, labor and preparation time while increasing sample consistency. The resulting FAMEs and Sterols can then be analyzed by GC/MS for quantification and isotopic enrichment analysis.

Method

There are three steps to metabolite analysis:

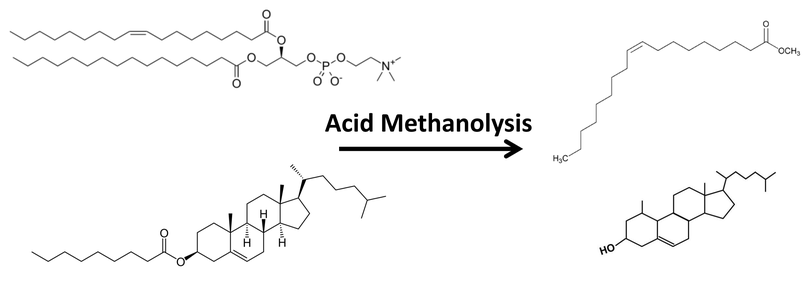
- **Sample collection and quantification**
- **Sample processing**
- **Sample analysis by MS**

Sample Collection



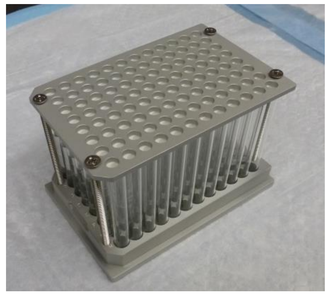
Sample Processing

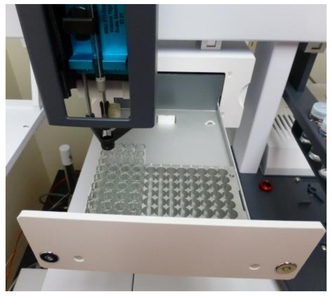
Acid methanolysis reaction breaks down complex lipids into fatty acid methyl esters and liberates cholesterol from cholesterol esters. (Ichihara K, Fukubayashi Y. Preparation of fatty acid methyl esters for gas-liquid chromatography. J Lipid Res. 2010 Mar;51(3):635-40)



Automation of Sample Preparation

- Add acid methanolysis mix w/ standards
- Incubate O/N at 45°C
- Add hexane/.04M NaCl
- Extract upper organic layer
- Dispense organics to 1.5mL tube





GC-MS Analysis

Samples Run on Agilent 7890B/5977A

- CTC PAL autosampler draws directly from 96 well rack
- Multimode inlet in solvent venting mode w/ large injection
  - No sample drying needed
- Midpoint column backflush reduces runtime to elution time of last analyte of interest.
  - Fatty acid and sterol GC-MS runs at 10 min. each

Cost Analysis

	Orig	HTS
10mL tube (2x)	$1.00	
robovial cap (2x)	$0.52	
pasteur pipette	$0.03	
2.5mL vial (1x)		$0.12
1.5mL vial (1x)		$0.10
plate liner (1/96x)		$0.03
toluene	$0.02	$0.00
methanol	$0.01	$0.00
HCl		
hexane	$0.02	$0.02
BSTFA+TMCS	$0.17	$0.03
pyridine	$0.02	$0.01
Total	$1.79	$0.32

Original Assay

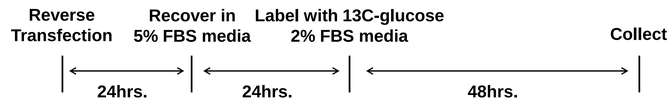
$1.79

HT Assay

$0.32

Assay Demonstration

Using this system, we can transfect cultured cells with SiRNAs and then culture them in ^{13}C-glucose to measure the amount of fatty acids and cholesterol that have been synthesized.



Relative De Novo Synthesis

	14:0	16:0	16:1	18:0	18:1	Chol.
SiCon	1.00	1.00	1.00	1.00	1.00	1.00
SiSCAP	0.79	0.74	0.35	0.85	0.42	0.57
SiSREBP1	0.97	0.99	0.89	0.99	0.82	1.98
SiELOVL6	0.91	0.80	0.85	0.66	0.64	0.85
SiLDLR	1.01	1.04	1.10	1.05	1.12	3.31
SiPDHA1	0.92	0.84	0.90	0.78	0.70	0.84

For further reading on isotopic labeling of lipids, GC-MS analysis and high-throughput sample preparation, see our lab's recent publications.

Limiting Cholesterol Biosynthetic Flux Spontaneously Engages Type I IFN Signaling. York AG, Williams KJ, Argus JP, Zhou QD, Brar G, Vergnes L, Gray EE, Zhen A, Wu NC, Yamada DH, Cunningham CR, Tarling EJ, Wilks MQ, Casero D, Gray DH, Yu AK, Wang ES, Brooks DG, Sun R, Kitchen SG, Wu TT, Reue K, Stetson DB, Bensinger SJ. Cell. 2015 Dec 17;163(7):1716-29.

Sterol regulatory element-binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity.
Kidani Y, Elsaesser H, Hock MB, Vergnes L, Williams KJ, Argus JP, Marbois BN, Komisopoulou E, Wilson EB, Osborne TF, Graeber TG, Reue K, Brooks DG, Bensinger SJ. Nat Immunol. 2013 May;14(5):489-99.

An essential requirement for the SCAP/SREBP signaling axis to protect cancer cells from lipotoxicity. Williams KJ, Argus JP, Zhu Y, Wilks MQ, Marbois BN, York AG, Kidani Y, Pourzia AL, Akhavan D, Lisiero DN, Komisopoulou E, Henkin AH, Soto H, Chamberlain BT, Vergnes L, Jung ME, Torres JZ, Liau LM, Christofk HR, Prins RM, Mischel PS, Reue K, Graeber TG, Bensinger SJ. Cancer Res. 2013 May 1;73(9):2850-62.

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