The Comparison of Lipid Extraction Methods for the Lipidomic Analysis of Blood Plasma

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INTRODUCTION

In the past 20 years, metabolomics has demonstrated an enormous potential in furthering the understanding of disease processes, phenotypic outcome of gene expression and biomarker discovery. Although metabolomics is and should remain an integrated approach by itself, its complexity requires analogous approaches focused on its components, such as lipidomics.

Whilst lipids in biofluids and tissues can be monitored by NMR spectroscopy without the requirement for extraction, this should be seen as only the first stage in lipidomic analysis. Subsequent analysis should then involve extraction of the lipids from the biofluid or tissue prior to further profiling using a multi-technique metabolomic approach involving both NMR spectroscopy and mass spectrometry.

AIM

Qualitative and quantitative comparison of different lipid extraction protocols and their reproducibility

METHODS

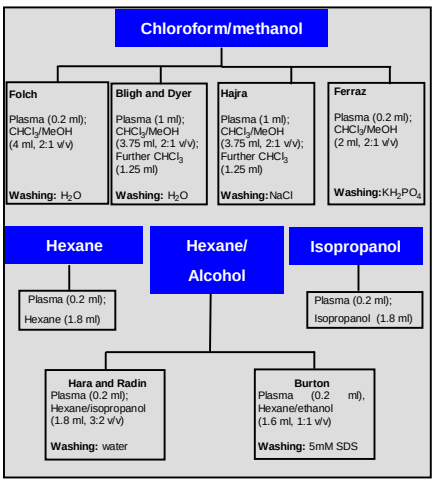

Fig. 1: The different extraction methods used in the comparison

All methods were carried out in triplicate and extracts profiled using a Bruker 500 MHz ^{1}H NMR spectrometer equipped with a cyroprobe, after reconstitution in deutrated chloroform with TMS as an internal standard.

RESULTS

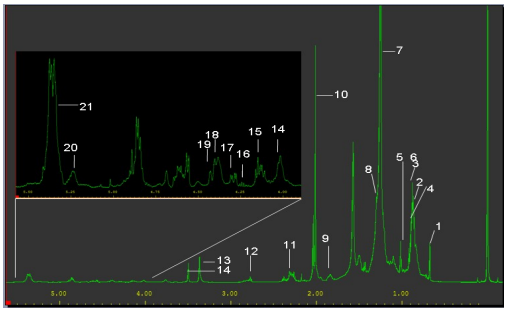

Fig. 2: 500 MHz ^{1}H NMR spectrum of plasma lipid extract obtained using the Folch extraction protocol

Table 1: Peak Assignments from Fig. 2

Peak	Chemical shift (ppm)	Assignment
1	0.67	-C18H_3 in total cholesterol
2	0.85	-C26H_3/-$C_{21}H_3$ in total cholesterol
3	0.86	CH_3 in fatty acyl chain
4	0.92	-$C_{21}H_3$ in free cholesterol
5	1.01	$C_{19}H_3$ in free cholesterol
6	1.02	-$C_{19}H_3$ in esterified cholesterol
7	1.25	-$(CH_2)_n$ in fatty acyl chain
8	1.29	$CHCH_2CH_2(CH_2)$- in fatty acyl chain
9	1.59	-CO-CH_2CH_2- in fatty acyl chain
10	2.01	-CH_2HC = in fatty acyl chain
11	2.28	-CO-CH_2- in fatty acyl chain
12	2.77	=CHCH_2CH = in fatty acyl chain
13	3.36	-$N^+(CH_3)_3$ in phosphotidylcholine (PTC) head group
14	3.78	-$CH_2N^+(CH_3)_3$ in PTC or sphingomyelin (SM) head group
15	4.01	>C_3H_2 in glycerol backbone of phospholipid (PL)
16	4.13	>C_1H_2 in glycerol backbone of PL and triacylglycerol (TAG)
17	4.29	-$CH_2CH_2N^+(CH_3)_3$ in PTC or SM head group
18	4.37	>C_1H_2 and =C_3H_2 in glycerol backbone of TAG
19	4.40	=C_1H_2 , in glycerol backbone of PL
20	5.23	C_2H in glycerol backbone of PL and TAG
21	5.36	-HC=CH- in fatty acyl chain

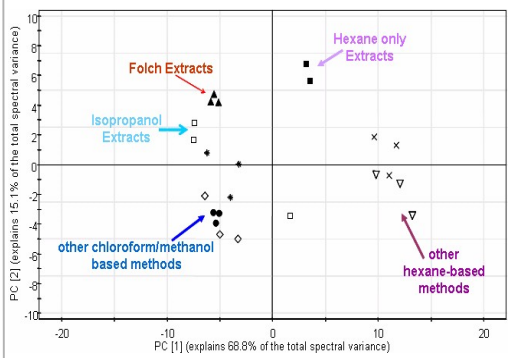

Fig. 3: Scores plot of PC1 vs PC2 highlights major differences in the lipids extracted when following different protocols

Table 2: Proteins retained by different methods were quantified because excessive levels of protein present in the samples will inhibit accurate absolute quantification of lipids by NMR and may cause additional difficulties when using extracts for LC-MS

Method	% total plasma protein retained
Folch	2.10
B-D	2.89
Hajra	0.59
Ferraz	0.97
IP	20.19
Hexane	0.00
H-R	0.59
Burton	0.00

Table 3 Lipids concentrations calculated in µmol/ml plasma

Peak	Folch	B-D	Hajra	Ferraz	IP	Hexane	H-R	Burton
1	2.9	2.1	2.3	5.7	2.7	0.8	2.4	4.6
2	3.1	1.2	1.2	4.3	2.4	---	---	18.1
3	7.8	2.1	2.2	18.4	5.9	27.5	20.4	27.6
4	3.2	1.4	1.5	6.8	3.4	---	2.8	4.4
5	1.7	0.9	0.7	2.3	2.4	---	1.8	2.6
6	3.7	2.1	2.0	5.2	2.8	1.1	2.9	4.5
7	219.8	53.4	56.1	281.0	164.8	2.6	107.9	67.6
8	34.3	14.3	21.4	63.7	23.3	279.5	18.9	24.4
9	68.9	17.1	18.4	82.0	72.6	50.4	57.2	58.3
10	8.6	2.5	2.6	11.5	6.8	---	4.8	8.9
11	9.3	6.2	6.1	18.1	7.2	---	6.3	10.8
12	3.1	2.1	2.2	6.4	2.4	---	2.1	2.7
13	4.5	3.1	3.1	9.6	3.0	1.8	2.0	0.3
14	1.4	0.8	0.9	2.5	1.1	---	0.7	---
15	1.1	0.8	0.8	2.4	1.7	0.3	0.8	0.2
16	1.1	0.7	0.7	2.5	0.7	---	0.6	0.6
17	---	0.4	0.4	1.3	0.4	---	0.3	---
18	6.1	3.4	3.0	16.4	10.7	7.1	8.1	4.4
19	1.6	0.5	0.5	1.8	0.5	0.1	0.5	---
20	1.5	1.1	1.1	3.3	1.3	---	0.9	0.6
21	7.5	5.2	5.1	15.1	6.1	1.6	5.5	8.3

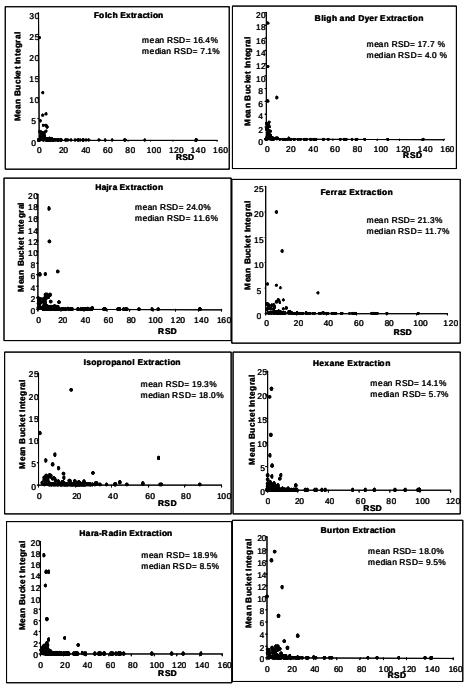

Fig. 4: Comparison of Method Reproducibility

CONCLUSIONS

The study demonstrates that lipid extraction methods vary greatly in their ability to extract different lipids.

The results highlight that the Ferraz method is the most efficient at extracting more lipids in higher quantities than all other methods. However, the RSD of this method is high.

Extraction with hexane is the least effective method for generic lipid extraction, probably due to its highly non-polar nature and inability to extract polar species.

Isopropanol produces surprisingly similar profiles to chloroform/methanol mixtures, but a lot of protein is retained and again, the RSD is high.