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The Science of Lysing

High-throughput lipid extraction for the analysis of human brain lipids

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CONTEXT

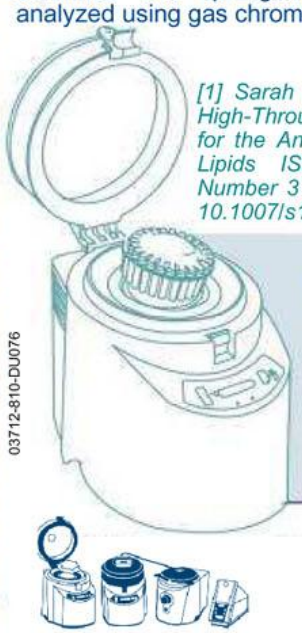
Traditional lipid extraction techniques are the bottleneck for modern shotgun **lipidomic studies**. To overcome this problem, protocol comparisons were made between the traditional Folch extraction (using chloroform and glass-glass homogenization) and a high-throughput method combining methyl-*tert*-butyl ether (MTBE) with mechanical homogenization (Precellys-24, Bertin Technologies) [1].

MATERIAL

- **Precellys 24.**
- Precellys lysing kit: CK14_0.5mL (03961-1-203).
- Samples: 10mg pulverized brain aliquot (human occipital cortex) weighted directly into the 0.5mL tube.
- Buffer: 300µL ice-cold methanol containing internal standards.

PROTOCOL

- Precellys 24: 6000 rpm, 2x30 sec.
- Lipids were extracted using MTBE or chloroform and analyzed using electrospray ionization mass spectrometry (ESI-MS; for phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, ceramide and sphingomyelin) and sterol species analyzed using gas chromatography (GC-MS).



[1] Sarah K. Abbott et al. An Improved High-Throughput Lipid Extraction Method for the Analysis of Human Brain Lipids, Lipids ISSN 0024-4201 Volume 48 Number 3 Lipids (2013) 48:307-318 DOI 10.1007/s11745-013-3760-z

RESULTS

No differences in lipid species composition were evident when the protocols were compared. From these studies we conclude that the high-throughput Bead-MTBE protocol is equivalent to the traditional Glass-Chloroform protocol (Folch) for lipid extraction and quantification of glycerophospholipid, sphingolipid and sterol species in human brain tissue.

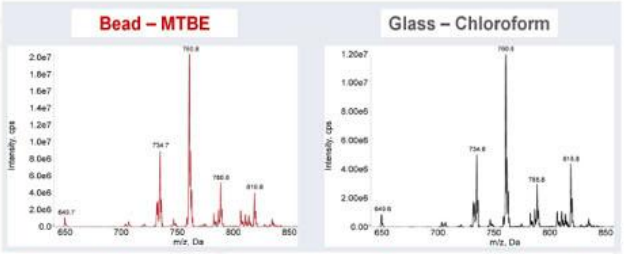


Figure 1: Representative spectra of human occipital cortex comparing Bead - MTBE to Glass – Chloroform (PC head group scan: precursor ion scan m/z 184.1)

This high-throughput Bead-MTBE protocol improves upon traditional lipid extraction methods as it is safer (less carcinogenic/toxic) and much more efficient. The Bead-MTBE protocol is approximately four times quicker than Glass-Chloroform in the homogenization of 24 samples (i.e.1 vs. 4 h), with the additional benefit being that tissue aliquots can be weighted directly into the Precellys tubes prior to homogenization (thus reducing double-handling times). The lower density of MTBE further enhances the lipid extraction procedure (by dissolving lipids in the upper phase) and is also better for the potential incorporation of robotics to further streamline lipidomic studies.

CONCLUSION

Lipidomic profiling of human brain tissue using MTBE extraction and mechanical bead homogenization with **Precellys** was comparable to traditional extraction techniques (i.e. chloroform extraction with glass-glass homogenization). The Bead-MTBE protocol provides an improved method for lipid extraction, as it is safer and much more efficient.

For more details, please contact precellys@bertin.fr

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