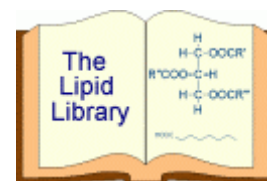


EXTRACTION OF LIPIDS FROM SAMPLES



Abstract: *The first task to be faced by an analyst when confronted by a new sample is the extraction of the lipids from the tissue matrix, and secondly to remove any non-lipid contaminants from the extract. It is probably viewed by most of us as the least interesting or rewarding of all our chores. In consequence, there is a danger of it being carried out hurriedly and incorrectly.*

Of the thousands of papers in the lipid literature that appear annually, very few deal with the topic of lipid extraction in depth, perhaps because the methodology is tedious and lacks interest *per se*. Many simple methods are available for extracting the bulk of the oils and fats from lipid-rich tissues, such as oil seeds, and direct extraction-analysis with supercritical fluids (reviewed by King [1]) is going to be used increasingly for specific lipids or types of sample, such as the fat-soluble vitamins, especially in the food industry. However, when analysts want to extract all the simple and complex lipids from a tissue in a near quantitative manner, they usually return to the "Folch" [2] or its variant the "Bligh & Dyer" [3] method.

I am not sure how it stands at present, but the Folch paper [2] was at one time one of the five most cited scientific publications of all time. Yet the method is often poorly understood and I suspect that very few of those who cite it have actually read the paper. (For example, in a high proportion of citations, the third author is incorrectly given a hyphenated surname). Nowadays, there is no excuse, as the paper is available from the *Journal of Biological Chemistry* as a free download.

Similarly, it seems to me that the Bligh & Dyer procedure has been used inappropriately on many occasions. Indeed, these notes were prompted by a publication [4], which suggested that this widely used method might be seriously flawed.

Folch Method

Lipids occur in tissues in a variety of physical forms, but the complex lipids are usually constituents of membranes, where they occur in close association with such compounds as proteins and polysaccharides, with which they interact by hydrophobic and van der Waals forces and perhaps by ionic bonds. Various solvents or solvent combinations have been suggested as extractants, but most lipid analysts use chloroform-methanol (2:1 by volume) as suggested by Folch *et al.* [2]. The endogenous water in the tissue is a ternary component of the system. The extract is shaken and equilibrated with one fourth its volume of a saline solution, when the mixture partitions into two layers, of which the lower is composed of chloroform-methanol-water in the proportions 86:14:1 (by volume) and contains virtually all of the lipids, while the upper phase consists of the same solvents in the proportions of 3:48:47 (by volume), respectively, and contains much of the non-lipid contaminants. It is not always recognised how important it is that the proportions of chloroform, methanol and water in the combined phases should be as close as possible to 8:4:3 (by volume), otherwise selective losses of lipids may occur. If carried out with the correct protocol, this method can give reliable results.

Bligh & Dyer Method

The Bligh and Dyer method [3] is a simple adaptation of the Folch procedure and was developed merely as an economical means (in terms of solvent volumes) of extracting lipids from tissues such as fish muscle, which contain relatively little lipid and a high proportion of water. It was not tested with any other type of sample by the authors, so they cannot be blamed if their method does not have universal applicability. The worrying aspect of the paper [4] alluded to above is that they also tested the method with fish tissues and found it lacking, not for the phospholipids where difficulties are frequently encountered with extraction procedures, but rather unexpectedly in the recovery of the non-polar lipids. However, Bligh and Dyer [3] do clearly state that for quantitative extraction of lipids, it is necessary to perform a re-extraction of the tissue residue with chloroform alone and add this extract to the filtrate prior to evaporation of the solvent. Of course, this would be expected to improve the yield of non-polar lipid. It is not evident that this was done in the new study [4].

Perhaps then, this old method is still viable after all - if you read the instructions! What is certain is that we can take no "standard" method for granted. Unfashionable and tedious tasks such as lipid extraction require to be re-investigated thoroughly by each new generation of lipid analysts. Chloroform-methanol may be the best lipid extractant, but it is certainly not the safest from environmental and health standpoints. I would like to see an exhaustive evaluation of hexane-isopropanol mixtures with a wide range of tissues, for example [5]. It obviously works well with rat liver, a standard tissue for biochemists, at least for the more abundant lipid classes. Does it work equally well with more intractable samples? Ethyl acetate/ethanol mixtures have been suggested also and require thorough independent testing [6].

Other Aspects of Extraction Methodology

Other aspects of lipid extraction and tissue handling must of course be carried out with care and require further investigation. Papers dealing with newer instrumental methods, including pressurized or accelerated solvent extraction and microwave irradiation or ultrasonification to improve yields show that these hold great promise, but they require to be tested on a wider range of sample matrices.

Biochemists must be aware that extraction should be carried out immediately after removal of tissues from living organisms, and if this is not possible, tissues should be stored in such a way that they do not deteriorate appreciably. Peroxidation can readily occur even at low temperatures [7]. The presence of a substantial amount of free fatty acids, diglycerides or phosphatidic acid in an extract is an infallible marker for poor technique. Plant tissues are best extracted with isopropanol to kill off lipolytic enzymes, for example. In an important but rather neglected paper, Kramer and Hulan [8] showed that animal tissues should be stored and extracted at -70°C to prevent artefact (especially free fatty acid) formation. Only a few of us may have access to such a facility, but it does perhaps confirm that we may have to treat the dreary preliminaries to lipid analysis more seriously.

References

1. King, J.W. [Supercritical fluid extraction: present status and prospects](#). *Grasas Aceites*, **53**, 8-21 (2002).
2. Folch, J., Lees, M. and Stanley, G.H.S. [A simple method for the isolation and purification of total lipides from animal tissues](#). *J. Biol. Chem.*, **226**, 497-509 (1957).
3. Bligh, E.G. and Dyer, W.J. [A rapid method of total lipid extraction and purification](#). *Can. J. Biochem. Physiol.*, **37**, 911-917 (1959).

4. Cabrini, L., Landi, L., Stefanelli, C., Barzanti, V. and Sechi, A.M. Extraction of lipids and lipophilic antioxidants from fish tissues - a comparison among different methods. *Comp. Biochem. Physiol.*, **101B**, 383-386 (1992).
5. Hara, A. and Radin, N.S. Preparation of lipid extracts from brain tissue. A rapid method of total lipid extraction and purification. Extraction of lipids and lipophilic antioxidants from fish tissues - a comparison among different methods. *Anal. Biochem.*, **90**, 420-426 (1978).
6. Lin, J.H., Liu, D.Y., Yang, M.H. and Lee, M.H. Ethyl acetate/ethyl alcohol mixtures as an alternative to Folch reagent for extracting animal lipids. *J. Agric. Food Chem.*, **52**, 4984-4986 (2004).
7. Whiteley, G.S.W., Fuller, B.J. and Hobbs, K.E.F. Lipid peroxidation in liver tissue specimens stored at subzero temperatures. *Cryo-Letters*, **13**, 83-86 (1992).
8. Kramer, J.K.G. and Hulan, H.W. A comparison of procedures to determine free fatty acids in rat heart. *J. Lipid Res.*, **19**, 103-106 (1978).

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