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PRACTITIONER'S REPORT

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Cofactors in Biota: results of a German interlaboratory exercise on the determination of total lipids in fish tissue

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Abstract The content of total lipids is a common cofactor necessary for a normalisation of organic contaminant concentrations in biological materials. Lipids are routinely determined gravimetrically after extraction of the material with chlorinated solvents. A new method substituting chlorinated solvents for a cyclohexane/isopropanol mixture was to be evaluated by an intercomparison exercise. The participating laboratories were requested to determine total lipid content in three different samples of fish tissue following their own procedures as well as the new method described in a standard protocol. No significant differences in the overall means of total lipid content were found for the investigated samples regardless of the determination method. Using the harmonised protocol of the new method, between laboratory reproducibilities below 10% were obtained in the exercise. The results indicate the applicability of the harmonised method for the determination of total lipids as a cofactor in the analyses of contaminant concentrations in marine biota samples.

Keywords Total lipids · Biota samples · Marine monitoring · Quality assurance · Intercomparison exercise

Introduction

Environmental monitoring programmes for organic contaminants must take into account their bioconcentration potential and their lipophilic properties. An evaluation of spatial distribution patterns and/or temporal trends of these substances in biota require information on the content of total lipids in the investigated samples for normalisation purposes. Currently, lipid determinations in biological tissue are routinely carried out after extraction with chlorinated solvents, mostly according to Bligh and Dyer [1].

A new method was developed by Smedes [2] for the determination of total lipid content in biological matrices using an isopropanol/cyclohexane mixture. A thoroughly validation of the method including an intercomparison exercise was part of the European project QUASH [3] which aimed at the development of quality assurance procedures of sampling and sample handling in marine environmental monitoring. According to the organisational structure of QUASH, international intercomparison exercises were followed by similar investigations on a national level.

To support the QUASH project, the Working Group on Quality Assurance in the German Marine Monitoring Programme (GMMP) decided to carry out a method evaluation exercise on the determination of total lipid content in three different types of fish tissue. The participating laboratories (Table 1) were requested to analyse the test materials for total lipid content following their own methods and the harmonised protocol of the Smedes method.

Table 1 List of participants

Federal Fisheries Research Centre, Institute of Fisheries Ecology, Hamburg
Federal Fisheries Research Centre, Institute of Biochemistry and Technology, Hamburg
Dr. Wiertz – Eggert – Dr. Jörissen GmbH, Laboratory for Trade and Environmental Protection, Hamburg
GSF-Research Centre for Environment and Health, Institute of Ecological Chemistry, Neuherberg
Institute of Bird Research, Ornithological Station Helgoland, Wilhelmshaven
State Agency for Veterinary and Food Investigations Mecklenburg-Vorpommern, Rostock
State Agency of Schleswig-Holstein for Food and Veterinary Investigations, Neumünster
State Veterinary Investigation Agency for Fish and Fish Products, Cuxhaven

Materials and methods

Test materials

Three fish tissues (plaice, mackerel, saithe) differing in water and/or lipid content (Table 2) were chosen for the purpose of the exercise. Bulk amounts of defrosted fish fillet (2.2–2.5 kg) were homogenised. Separate test portions were manually filled in tins and semi-automatically closed. All test portions were heat sterilised at 108 °C.

A test of homogeneity was carried out following a procedure recommended by Thompson and Wood [4]. It was found that the test materials were sufficiently homogeneous for the intended use.

The prepared test portions were stored at room temperature and proved to be stable during the time frame of the exercise.

Intercomparison exercise

The participants (Table 1) were requested to carry out triplicate determinations of total lipid content in the test materials by both their routine in-house methods and the Smedes procedure. An evaluation of the returned method questionnaires showed that several methods are in use in the laboratories varying in solvent composition and extraction steps [1, 5–8]. This part of the intercomparison test can be regarded as a cooperative trial in which the comparability of different methods for a single parameter is evaluated. In the other part of the exercise, all laboratories followed strictly the detailed description of the Smedes method [2], which was distributed together with the announcement of the exercise. Briefly, total lipids were to be extracted using a cyclohexane/isopropanol mixture before their gravimetrical determination. The original data reported by the participants are shown in Table 3 and Table 4. Additionally, the dry matter content was to be determined in separate subsamples of the test portions.

Table 2 Test materials selected for the intercomparison exercise and summary statistics of dry matter and total lipid content determination

Test Material		Plaice (*Pleuronectes platessa*)	Mackerel (*Scomber scombrus*)	Saithe (*Pollachius virens*)
Sample Code		BT01	BT02	BT03
Test Portions	Number	29	30	30
	Average weight [g]	76	75	76
Dry Matter Content	Number of results	50	50	50
	Number of outliers[a]	1 (C)	–	–
	Mean [%]	19.27	25.92	19.70
	S_r[b] [%]	0.85	0.98	0.64
	S_R[c] [%]	1.70	1.59	1.11
Total Lipids (in-house method)	Number of results	28	29	29
	Number of outliers[a]	1 (C)	–	1 (B), 1 (C)
	Mean [%]	1.015	3.493	0.909
	S_r[b] [%]	3.17	3.56	5.61
	S_R[c] [%]	17.49	13.21	17.89
Total Lipids (*Smedes* method)	Number of results	24	24	24
	Number of outliers[a]	1 (A)	–	–
	Mean [%]	0.982	3.519	0.915
	S_r[b] [%]	4.31	2.55	5.34
	S_R[c] [%]	8.39	5.79	8.82

[a]Outliers – see text for an explanation of the type of outlier rejected.
[b]S_r: repeatability (relative within laboratory standard deviation).
[c]S_R: reproducibility (relative between laboratory standard deviation).

Table 3 Raw data of the intercomparison exercise on the determination of total lipids in fish tissue – in-house method

Test Material	Sample Code	Laboratory	Result 1	Result 2	Result 3
Plaice	BT01	AH	1.18	1.11	1.21
Mackerel	BT02	AH	4.03	4.04	3.95
Saithe	BT03	AH	1.08	1.20	1.08
Plaice	BT01	BH	0.890	0.936	0.875
Mackerel	BT02	BH	3.652	3.633	3.625
Saithe	BT03	BH	0.812	0.746	0.768
Plaice	BT01	CH	0.8163	0.8295	0.8080
Mackerel	BT02	CH	2.155	2.569	2.539
Saithe	BT03	CH	0.9202	0.9069	0.9008
Plaice	BT01	CB	1.200		
Mackerel	BT02	CB	3.958		
Saithe	BT03	CB	1.051		
Plaice	BT01	DH	0.934	1.073	1.134
Mackerel	BT02	DH	3.694	3.906	3.718
Saithe	BT03	DH	1.069	1.131	0.937
Plaice	BT01	EH	1.200	1.244	1.217
Mackerel	BT02	EH	3.680	3.775	3.776
Saithe	BT03	EH	0.982	1.065	1.069
Plaice	BT01	FH	1.115	1.123	1.115
Mackerel	BT02	FH	3.423	3.154	3.194
Saithe	BT03	FH	0.912	0.935	0.891
Plaice	BT01	GH	1.100	1.200	1.200
Mackerel	BT02	GH	3.500	3.700	3.900
Saithe	BT03	GH	1.000	1.200	1.300
Plaice	BT01	HH	0.8555	0.8381	0.8608
Mackerel	BT02	HH	3.4529	3.3866	3.4265
Saithe	BT03	HH	0.6869	0.6908	0.7155
Plaice	BT01	IH	0.7910	0.7910	
Mackerel	BT02	IH	3.3016	3.4260	3.4000
Saithe	BT03	IH	0.7685	0.7284	0.6831
Plaice	BT01	IO	0.8731		
Mackerel	BT02	IO	3.3333		
Saithe	BT03	IO	0.3550		

Table 4 Raw data of the intercomparison exercise on the determination of total lipids in fish tissue – Smedes method

Test Material	Sample Code	Laboratory	Result 1	Result 2	Result 3
Plaice	BT01	AS	0.99	0.99	0.97
Mackerel	BT02	AS	3.63	3.65	3.65
Saithe	BT03	AS	0.91	0.91	0.91
Plaice	BT01	BS	0.898	0.998	0.853
Mackerel	BT02	BS	3.672	3.561	3.559
Saithe	BT03	BS	0.797	0.728	0.873
Plaice	BT01	CS	1.005	0.9960	1.109
Mackerel	BT02	CS	3.362	3.334	3.312
Saithe	BT03	CS	0.9173	0.9152	0.9187
Plaice	BT01	DS	0.988	0.917	0.892
Mackerel	BT02	DS	3.706	3.884	3.773
Saithe	BT03	DS	0.878	0.917	0.894
Plaice	BT01	FS	0.909	0.942	0.967
Mackerel	BT02	FS	3.441	3.372	3.399
Saithe	BT03	FS	0.939	0.941	0.928
Plaice	BT01	GS	0.900	0.900	2.100
Mackerel	BT02	GS	3.000	3.300	3.300
Saithe	BT03	GS	0.800	1.000	0.800
Plaice	BT01	HS	0.9897	0.9901	0.9905
Mackerel	BT02	HS	3.5146	3.6067	3.6207
Saithe	BT03	HS	0.9541	0.9517	0.9608
Plaice	BT01	IS	1.1549	1.1134	1.1168
Mackerel	BT02	IS	3.7530	3.5075	3.5551
Saithe	BT03	IS	1.0526	1.0388	1.0372

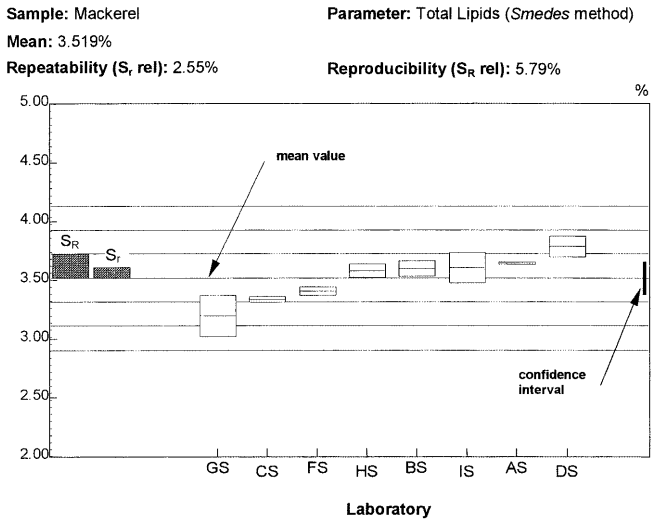

Fig. 1 Example for the data assessment of the intercomparison exercise (sample BT02, Smedes method)

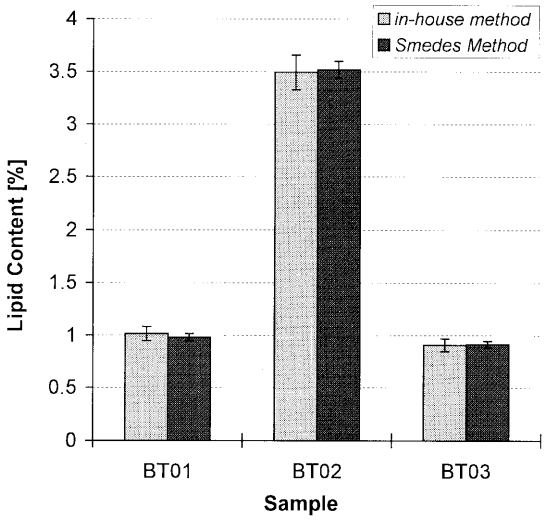

Fig. 2 Comparison of results between Smedes method and the in-house methods routinely used by the participating laboratories (error bars are confidence intervals of the overall means, $P=5\%$)

The data assessment followed the ISO 5725–2 protocol [9] implemented in the software package PROLAB98 (quo data, Dresden, Germany). Several tests of outliers had been carried out before repeatabilities and reproducibilities of total lipid determinations were calculated. The following types of outliers were rejected:

- Type A: individual within laboratory outlier (e.g., one out of three results deviated significantly from the mean value; Grubbs' test, P=1%),
- Type B: between laboratory outlier due to significant deviation of the laboratory's mean from the overall mean (Grubbs' test, P=1%), and
- Type C: between laboratory outlier due to significant difference between the within laboratory standard deviation and the mean of all within laboratory standard deviations (Cochran's test, P=1%).

After elimination of outliers (Table 2), the within laboratory means, the within laboratory standard deviations and the coefficients of variation were calculated (Fig. 1).

Results

The low reproducibility values and the small number of outliers indicated that all participating laboratories were capable of carrying out accurate dry matter determinations in fish tissue (Table 2). The reproducibility values for all three investigated samples were in good agreement with those obtained by the international QUASH exercise [10]. As in that exercise, the small reproducibilities of the dry matter results were an indication of a sufficient homogeneity as well as of a successful rehomogenisation of the samples by the participating laboratories.

Repeatabilities of the total lipid determination between 2% and 6% were obtained for both the in-house methods and the Smedes method. However, the comparability (reproducibility) of the methods routinely used by the participating laboratories was between 13% and 18% due to differences in the procedural steps. Following the harmonised procedure of the Smedes method, the reproducibility of the total lipids determination decreased to values between 6% and 9%.

In analysing results of interlaboratory studies, Horwitz [11] found a relation between the random error and the analyte concentration that could be expressed by a simple exponential function. The variability among laboratories using the Smedes method (a collaborative study, in which the procedure was specified at all steps in considerable detail) was in acceptable accordance with this equation and indicative of achievable and acceptable performance of the method by the participating laboratories. When the laboratories used their own favoured in-house methods (a cooperative study), reproducibilities deviated significantly from the expected values. In this case, significant differences between solvent properties, procedural steps and equipment created systematic variations of lipid extracts peculiar to each laboratory. This systematic variation became a component of the between laboratory variability, which exceeded markedly the value derived from the Horwitz-function.

Fig. 2 shows the overall means and confidence intervals of the investigated samples obtained by both total lipid determination methods. No significant differences were found between the total lipid values obtained by the different methods.

The results of the intercomparison exercise speak for an overall good proficiency of the participating laboratories and for the applicability of the new Smedes method for the determination of total lipids in biota samples. However, further investigations using other tissues with varying lipid content are needed to replace the common total lipid determination procedures by a method using non-chlorinated solvents.

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