

QUASH - WP2

„Lipid and Water as Cofactors“

Report on a German Interlaboratory Study

„Determination of Dry Matter and Total Lipids

in Fish“

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1. INTRODUCTION

Within the framework of the QUASH WorkPackage 2 („Determination of cofactors in biota“) a new method for the determination of lipids using non-chlorinated solvents (the so-called *Smedes* method) was to be evaluated. According to the organisational structure of QUASH, international intercomparison studies should be 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 for total lipid determinations in three different types of fish tissue (test materials). The test materials were prepared by the Federal Fisheries Research Centre in Hamburg. The participating laboratories were requested to determine the dry matter content of the test materials and to carry out total lipid determinations following their own methods (cooperative study) as well as the method of *Smedes* (collaborative study). This report summarises the results of both studies and gives information on the repeatabilities and reproducibilities obtained.

2. TEST MATERIALS

2.1. Preparation

Three fish tissues differing in water and/or lipid content (Plaice, Mackerel, Saithe) were chosen for the purpose of the exercise. The test materials were provided by the Federal Fisheries Research Centre, Hamburg. Bulk amounts of defrosted fish filet (2.2 - 2.5 kg) were homogenized. Separate test portions were manually filled in tins and semi-automatically closed. All test portions were heat sterilised at 108°C. Table 1 gives information on the test materials for the method evaluation study.

Table 1: Test materials selected for the exercise

Test Material	Sample Code	Test Portions	
		Number of canned tins	Average weight per tin [g]
Plaice (<i>Pleuronectes platessa</i>)	BT01	29	76
Mackerel (<i>Scomber scombrus</i>)	BT02	30	75
Saithe (<i>Pollachius virens</i>)	BT03	30	76

2.2. Homogeneity

A test of homogeneity was carried out at the Federal Fisheries Research Centre following a procedure recommended by Thompson and Wood /3/. It was found that the test materials were sufficiently homogeneous for the intended use.

2.3. Stability

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

3. INTERCOMPARISON STUDY

3.1. Organisation

The distribution of the test materials to the participating laboratories and the data evaluation was carried out by the German Federal Environmental Agency. Table 2 shows the time schedule of the exercise. The participating laboratories are listed in Annex 1. The test portions of the samples BT01, BT02, BT03 were sent off to 9 laboratories together with data report sheets and a method description questionnaire (see Annex 2). The Federal Environmental Agency received analytical results of 8 laboratories until deadline (see Table 2). Despite the delayed data reporting of laboratory I, its results were incorporated in the data assessment and evaluation.

Table 2: Time schedule of the exercise for the determination of the total lipids and dry matter in fish

Date	Action
December 1998	Announcement of the intercomparison exercise by the Working Group on Quality Assurance in the GMMP
January 1999	Preparation of test materials (three different fish tissues)
February 1999	Distribution of the samples to the participants
31.03.1999	Deadline for submitting of analytical results
May/June 1999	Evaluation of results/Report

3.2. Analytical Procedures

The participants were requested to carry out five replicate determinations of dry matter using their own ('home') procedures. Total lipid contents of the test materials were to be analysed in triplicate using both the laboratory's 'home' methods and the procedure according to the protocol of *Smedes*, respectively. A description of the *Smedes* method was distributed to all laboratories in connection with the announcement of the exercise. Annex 3 gives a compilation of the procedures used for dry matter and total lipid determination.

3.3. Data Assessment

The data assessment followed the ISO 5725-2 protocol implemented in the software package PROLAB98 (Dr. Uhlig, quo data, Dresden), which is routinely used at the Federal Environmental Agency for the evaluation of laboratory proficiency tests. Table 3 shows the different steps of the assessment. The original data of all participants are summarised in Annex 4.

Table 3: Data assessment for an evaluation of the lipid determination exercise

Step	Laboratory
Coding of participating laboratories	A - I
Separation of multiple data sets of individual laboratories (Coding of different methods for total lipid determination)	F1/F2 XH or XS
Creating an Excel spread sheet of all data	
Twofold comparison of the created database with the original data	
Import of the data into the software package PROLAB98 for statistical evaluation	
Evaluation of the data according to ISO 5725-2	

Several tests of outliers had been carried out before the repeatability and the reproducibility of the dry matter and total lipid determinations were calculated. According to ISO 5725-2, the following types of outliers were rejected:

- Type A: individual within laboratory outlier (e.g. one out of three or five, respectively, results deviates significantly),
- Type B: between laboratory outlier due to significant deviation of the laboratory's mean from the overall mean,
- Type C: between laboratory outlier due to significant difference between the within laboratory standard deviation and the mean of all within laboratory standard deviations.

After elimination of outliers, the within laboratory means, the within laboratory standard deviations and the coefficients of variation were calculated. A graphical presentation of the analytical results for the samples BT01, BT02 and BT03 and the calculated values of the overall mean, the within laboratory standard deviation (repeatability) and the between laboratory standard deviation (reproducibility) for all parameters are given in Annex 5. Outliers are indicated with the above mentioned letters.

Table 4 summarises the overall statistics of the dry matter and total lipid determination exercise. Since the reproducibility SR can be regarded as the between laboratory comparability, all participating laboratories are capable of carrying out accurate dry matter determinations in fish tissue. The calculated reproducibility values of about 1.5% for all three investigated samples are in good agreement with those obtained by the international QUASH exercise /1/. As in that exercise, the small between laboratory differences in the dry matter results are an indication of a sufficient homogeneity of the samples as well as of a successful rehomogenisation of the samples by the participating laboratories.

Compared to the international round, similar repeatability and reproducibility values were obtained for the determination of total lipids in the German exercise. Repeatabilities between 2% and 5% were obtained for both the home methods and the *Smedes* method. However, the comparability of the total lipid determination methods routinely used by the participating laboratories is between 13% and 18% due to differences in the procedural steps (see Annex 3). Following the harmonised procedure of the *Smedes* method, the reproducibility of the lipid determination drops down to values between 6% and 9% (see 3.4).

In analysing results of interlaboratory studies, Horwitz /2/ found a relation between the random error and the analyte concentration that could be expressed by a simple exponential function:

$$\text{RSD}_R (\%) = 2^{(1-0.5\log C)} .$$

where $\text{RSD}_R (\%)$ denotes the relative standard deviation among laboratories (reproducibility), and C is the analyte concentration expressed in mass/mass units. The variability among laboratories using the *Smedes* method (a collaborative study, in which the procedure was specified at all stages in considerable detail) was in acceptable accordance with this equation and indicative of achievable and acceptable performance of the method by the participating laboratories (Table 4). When the laboratories used their own favoured 'home' methods (a cooperative study), reproducibilities deviated significantly from the expected values (Table 4). 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 afore-mentioned function.

Table 4: Summary statistics of dry matter and total lipid determination intercomparison exercise

Dry Matter			
Sample Code	Mean [%]	si* [%]	SR** [%]
BT01	19.27	0.85	1.70
BT02	25.92	0.98	1.59
BT03	19.70	0.64	1.11

Total Lipid Content ('home' method)			
Sample Code	Mean [%]	si* [%]	SR** [%]
BT01	1.015	3.17	17.49
BT02	3.493	3.56	13.21
BT03	0.909	5.61	17.89

Total Lipid Content (<i>Smedes</i> method)			
Sample Code	Mean [%]	si* [%]	SR** [%]
BT01	0.982	4.31	8.39
BT02	3.519	2.55	5.79
BT03	0.915	5.34	8.82

*si - repeatability (relative within laboratory standard deviation)

**SR - reproducibility (relative between laboratory standard deviation)

3.4. Comparison of the Total Lipids Determination Methods

As described in the data assessment section, the results of the cooperative study to evaluate the 'home' methods of all laboratories indicate the comparability of these methods despite of different procedural steps (extraction schemes, solvents used etc., see Annex 3). Therefore, all 'home' method data could be pooled, and the overall mean, the overall standard deviation and the confidence interval ($\alpha = 0.05\%$) were calculated. The same parameters were determined for the *Smedes* method data. Figure 1 shows the overall means and confidence intervals of the investigated samples obtained by both total lipid determination methods. Because of the different reproducibilities, a *t-Test* for the overall means comparing the 'home' methods and the *Smedes* method was carried out with separate variance estimates and approximate degrees of freedom (Software package Statistica 5.0). No significant differences were found between the total lipid values of the investigated samples (see Figure 1). However, further investigations using other tissues with varying lipid content are needed to replace the common total lipid determination procedures by a method without the use of chlorinated solvents.

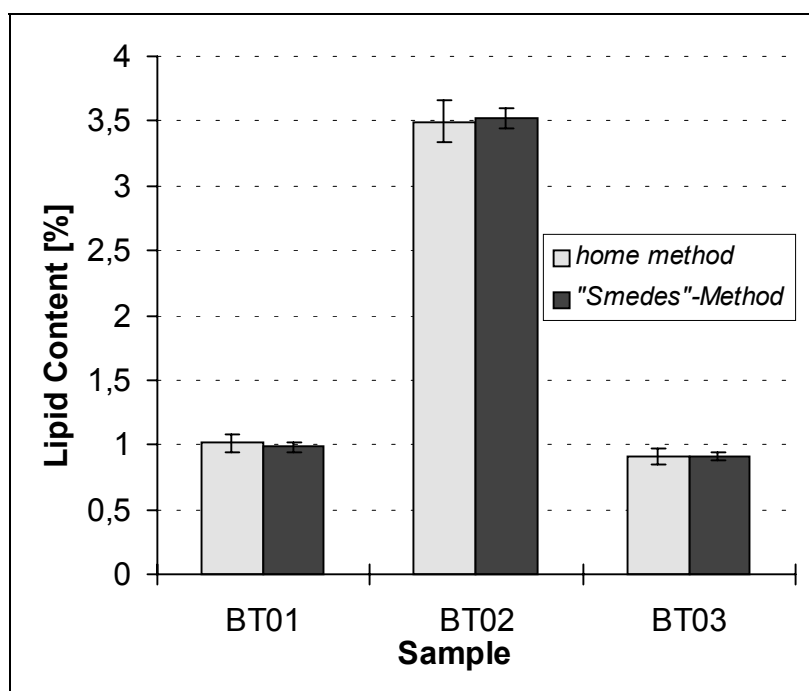


Figure 1: Comparison of the results of the new *Smedes* method and of methods routinely used by the participating laboratories (the error bars are confidence intervals of the overall means, $\alpha = 0.05\%$)

3.5 Laboratory Evaluation

An evaluation of the laboratory's proficiency to determine dry matter and total lipid contents in fish tissue was carried out using z-scores according to IUPAC /3/. Since the PROLAB98 package does not allow the use of target standard deviations, the z-scores were calculated using the between laboratory standard deviation. The z-scores of all laboratories are given in Annex 6. Regarding the dry matter determination and the results of the *Smedes* method, all laboratories obtained satisfactory z-scores ($z < 2$) for all investigated samples. Additionally, the between laboratory standard deviation is better than the target variance of 6% set by QUASH /1/ for the determination of cofactors in biota. These results speak for an overall good proficiency of the laboratories and for an applicability of the new *Smedes* method for the determination of total lipids. Almost all of the laboratories meet the proficiency criteria when using their 'home' methods for the determination of total lipids. However, the calculated reproducibilities exceed the target value of 6% for all investigated samples (see 3.3).

4. ACKNOWLEDGEMENT

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5. REFERENCES

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