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ALTERNATIVE SULPHURIC ACID PRE-TREATMENT AND SILICA BASED SOLVENT CHANGE DEMONSTRATED FOR LARD AND FISH OIL ANALYSED BY EROD-BIOASSAY

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Introduction

Bioassays for routine application should be simple and robust to use. Some samples tend to plug[1] the clean up column. Aim of the study was to develop a solution without further equipment or chemicals. Concepts of column clean up with and without pre-treatment were compared.

Materials and methods

Aliquots of lard and bovine fat were spiked with 20 µl 2,3,7,8-TCDD/PCB-126 (1/10 w/w) in DMSO. Every subsample with its spike was freshly prepared 6fold. Fish oil was tested without spike. Original samples were as well analysed via HRGC/HRMS. 2,3,7,8-TCDD and PCB-126 were from LGC-Standards. The quality of all other chemicals, the preparation of solutions and the EROD-bioassay (PBS/dispense) using rat hepatoma cells (H4IIEC/T3) criteria were described before[2,3,4,5]. The clean-up procedure depended on the tested amount of fat, see table 1.

Basic column: The clean up for 1 g bovine fat[3,6] was scaled up for 2 g lard.

"Dry" sulphuric acid pre-treatment:
3 g lard were spiked and solved in 5 ml n-hexane. 25 ml acidic silica (44 w% H_2SO_4) were added and mixed with the fat. This step increased the recovery of spikes due to less displacement of polar DMSO by nonpolar n-hexane (data not shown). After two to five minutes 15 ml n-hexane were added and agitated. Some of the solvent evaporated so the consistence of the mixture allowed quickly to pour the whole blend on top of the corresponding clean up column.

Silica based solvent change
For fish oil a coupling of fat extraction and acidic treatment preceding the cleanup column was tested. Lipids were extracted by adding Na_2SO_4 and eluting with 200 ml n-hexane/acetone 2/1 (v/v)[3,4,7]. Fat extracts were reduced, transferred with 5 ml n-hexane/acetone 2/1 (v/v) to 10 ml heated silica and mixed. Solvents were removed by evaporation. The dry blend was mixed with 15 ml acidic silica (44 w% H_2SO_4). The mixture was moistened by 15 to 20 ml n-hexane, mixed again and poured onto a clean up column as described for 3 g lard.

Results and discussion

The comparison of data for bovine fat to the larger column for 2 g lard is shown in table 2. The curve parameters show similar values. Most authors[2,6,8] use silica clean up of comparable acid to fat ratio. Our results for basic columns fit well into the scheme. They lead to the assumption of similar dose-response-curves for fat from different mammals. Further data must be gained to verify which kinds of fat can be gathered to matrix groups in routine analysis.

Figure 1 compares 2 g lard cleaned up on basic columns and 3 g lard with preceding acidic silica treatment respectively. Samples of identical concentration (0.1; 1.1; 2.1 pg BEQ/g fat) show no significant difference (p=0.05) between the concepts. The curve parameters are almost identical. Other authors use acidic pre-treatment with considerable amounts of n-hexane[1,9]. Liquid sulphuric acid[1] or loose bulk acidic silica[9] are used. The advantages of the concepts are discussed in table 3.
In a following step the concept of "dry" silica pre-treatment was tested for fish oil (1 g each) and for some samples coupled to fat extraction as described above. No significant differences (p=0.05) could be observed between extracts obtained by different extraction and clean up combinations, s. table 4.
By using "dry" silica based sulphuric acidic pre-treatment samples with lipophilic compounds not soluble in n-hexane can be addressed. Further it is possible to pre-treat samples that tend to plug the clean

up column like rancid oil. A transfer of polar lipids to the cleanup is possible without acetone while washing[1] is avoided. No change in solvent composition or devices used so far was necessary. Depending on the amount of fat the concept can be scale up. When working with DMSO-spikes the use of glassware is strongly recommended. For naturally contaminated fish oil vessels from polypropylene obtained no negative effect.

References:

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Alternative sulphuric acid pre-treatment and silica based solvent change demonstrated for lard and fish oil analysed by EROD-bioassay

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Abstract attach: Tables and figures

Table 1: Set up of columns for clean-up. Silica was added by measuring spoon, with 8 ml representing 5 to 5.5 g

Column set up	**≤ 1 g fat**[3,6]	**≤ 2 g fat**	**≤ 3 g fat**
top	3 g Na_2SO_4	3 g Na_2SO_4	Mixture from pre-treatment
H_2SO_4 on silica	8 ml 22 w% H_2SO_4 8 ml 33 w% H_2SO_4	16 ml 22 w% H_2SO_4 16 ml 44 w% H_2SO_4	16 ml 44 w% H_2SO_4
bottom	3 g Na_2SO_4 on glass wool		
Conditioning of column before addition of samples			
n-hexane	40 ml	100 ml	40 ml
Application of sample	solved in 2 to 5 ml n-hexane		pouring of mixture
Elution of sample after rinsing of the sample vessels			
n-hexane	2 x 5 ml + 28 ml	2 x 5 ml + 140 ml	2 x 5 ml + 65 ml

Table 2: Validation of spiked fat, each concentration was extracted 6fold and measured via EROD-bioassay. Mean values of bioassay with coefficients of variation (CV), curve parameters and HRGC/HRMS results are shown.

Bovine fat 1 g, basic column[3]		**Lard, 2 g fat, basic column**	
HRGC/HRMS pg WHO-TEQ[5]/g fat (Σ PCDD/F +dl PCB)	EROD pg BEQ/g fat ± CV %	HRGC/HRMS pg WHO-TEQ[5]/g fat (Σ PCDD/F +dl PCB)	EROD pg BEQ/g fat ± CV %
-	-	0.14	0.10 ± 56.6 %
-	-	0.89	0.73 ± 17.6 %
-	-	1.14	1.20 ± 11.0 %
2.88	3.01 ±8.60 %	1.64	1.58 ± 11.0 %
4.84	5.15 ±18.0 %	2.14	2.24 ± 16.0 %
6.84	6.85 ±8.40 %	3.14	3.58 ± 14.3 %
8.88	9.78 ±15.1 %	4.14	4.16 ± 13.5 %
Parameter of linear curve	$y = 1.15x - 0.49$ $R^2 = 0.95$	Parameter of linear curve	$y = 1.07x - 0.09$ $R^2 = 0.94$

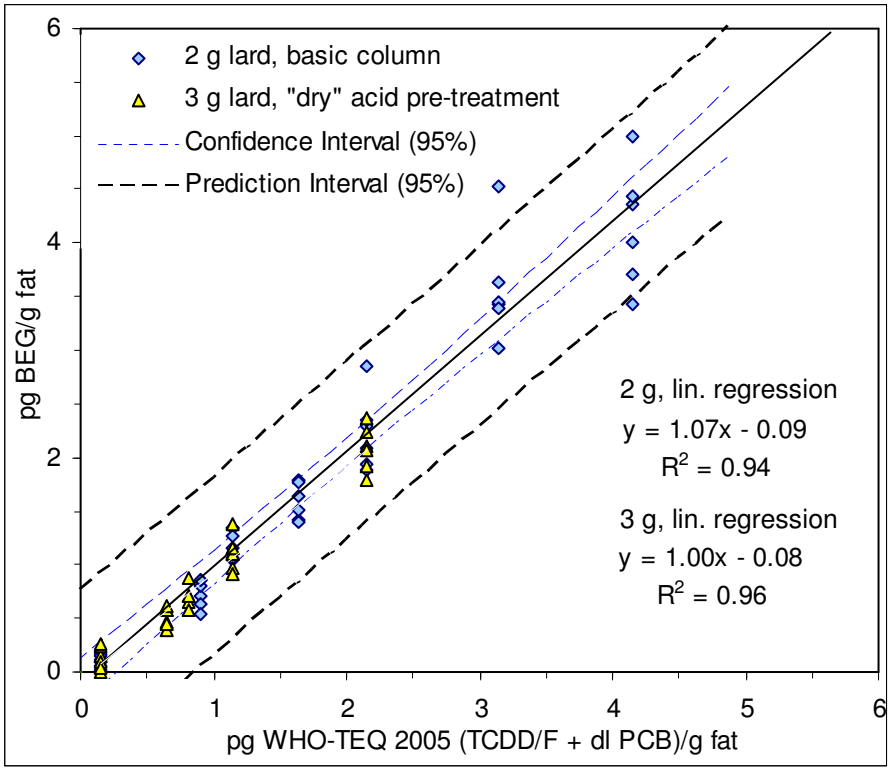

Figure 1: Validation of 2 g lard on basic columns and for 3 g lard with acidic silica pre-treatment. Each concentration was extracted 6fold and measured via EROD-bioassay. One point represents the mean of three wells. Results are based on WHO-TEF[5]. Confidence and prediction intervals for 2 g lard, curve parameters for linear fit and coefficients of determination (R^2) are shown.

Table 3: Comparison of acidic pre-treatment concepts

Concept	**Amakura[1]**	**US EPA[9]**	**this study**
sample	30-50 g fast food	5 g soil/sediment	≤ 3 g fat
visible n-hexane phase	✓	✓	-
oxidation	30-50 ml H_2SO_4 conc.	H_2SO_4 on silica small amount	25 ml 44 w% H_2SO_4 on silica
standard mixing glass ware	✓	tight and inert sealing	✓
scaling possible	✓	✓	✓
optical control	clear n-hexane-phase		browning
load slurry* on column	-	✓	✓
specific needs	washing steps prevention of charring	shaker	-

* sample mix with acidic silica after oxidation

Table 4: Comparison of different extraction and clean-up schemes for 1 g fish oil

Group	Number of samples n	Fat Extraction	Sulphuric acid Pre-treatment	pg BEQ/g fat ± CV %
A - control	16	-	-	1.62 ± 23.9%
B	12	-	✓	1.64 ± 9.5%
C	4	✓	✓	1.66 ± 11.4%