Microwave extraction of organic substances minimize the solvent consumption

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Luca Rota

Milestone Product Specialist

EXTRACTION WITH ETHOS X

EXTRACTION OF POPs
IN ENVIRONMENTAL
SAMPLES (MAE)

FAT EXTRACTION
IN FOOD SAMPLES

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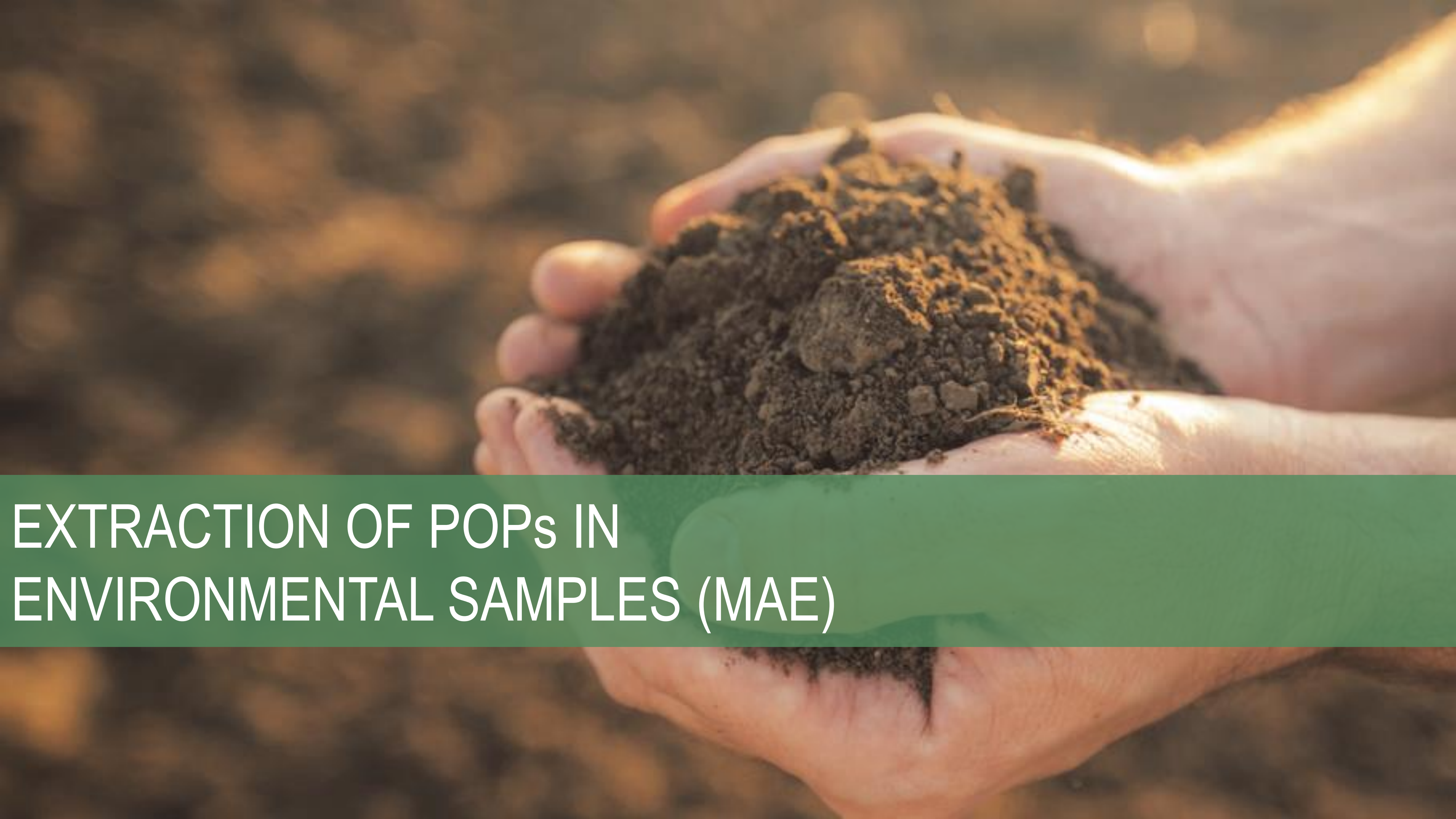
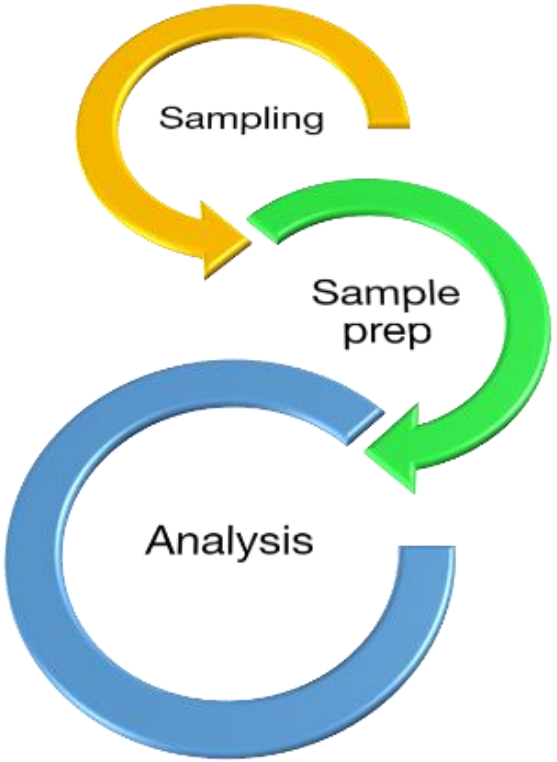


EXTRACTION OF POPs IN ENVIRONMENTAL SAMPLES (MAE)

CHALLENGES OF ENVIRONMENTAL LABORATORIES

- Fast delivery of results
- Low cost analyses
- Sometimes unpredictable weekly work-load
  - Soil remediation campaigns
  - Large samples batches
- Wide range of different matrices (cross contamination)
- Safety concern for some solvents (chlorinated)



Often, sample preparation is the bottleneck

OTHER TECHNIQUES

EPA method 3540 - Soxhlet extraction- introduced in 1880

EPA method 3550C
Ultrasonic extraction-1980

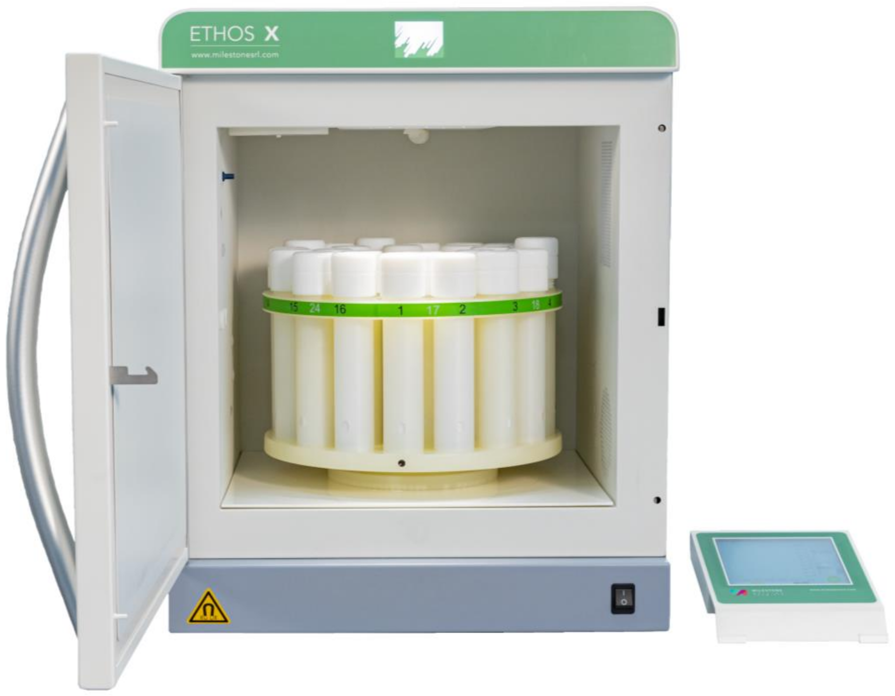
EPA method 3545A=Press. Liquid extraction (PLE)- Mid 1990s

Common drawbacks:

- Not suitable for all environmental samples (mixed terrains)
- High solvent consumption
- Long extraction time
- Cumbersome cleaning step
- Memory or carryover effect
- High initial investment (PFE)

MICROWAVE ASSISTED EXTRACTION (MAE)

- MAE is a well-established technique for extracting contaminants from solid and semi-solid samples with liquid solvents
- MAE uses high temperature and pressure with common solvents to increase the efficiency of the extraction process.



US EPA 3546 METHOD

METHOD 3546

MICROWAVE EXTRACTION

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed Standard Operating Procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

1.0 SCOPE AND APPLICATION

1.1 This method is a procedure for extracting water insoluble or slightly water soluble organic compounds from soils, clays, sediments, sludges, and solid wastes. This method was developed and validated on commercially-available solvent extraction systems. Its procedure uses microwave energy to produce elevated temperature and pressure conditions (i.e., 100 -

US EPA 3546 METHOD

Samples

- Soils
- Clays
- Sediments
- Sludges
- Solid wastes

Organic compounds

- Chlorinated pesticides
- Semivolatile organics
- PAHs
- PCBs
- Chlorinated herbicides
- Phenols
- Organophosphorus pesticides
- Dioxins and furans

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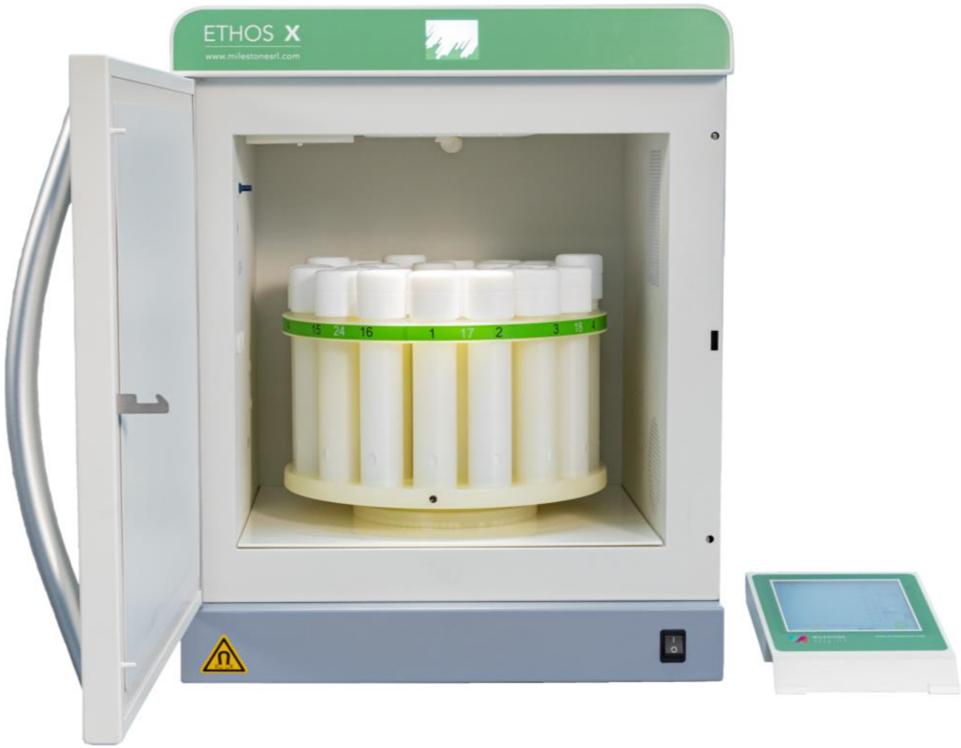
HARDWARE

POPs - ETHOS X HARDWARE



Ethos X

fastEX-24
rotor

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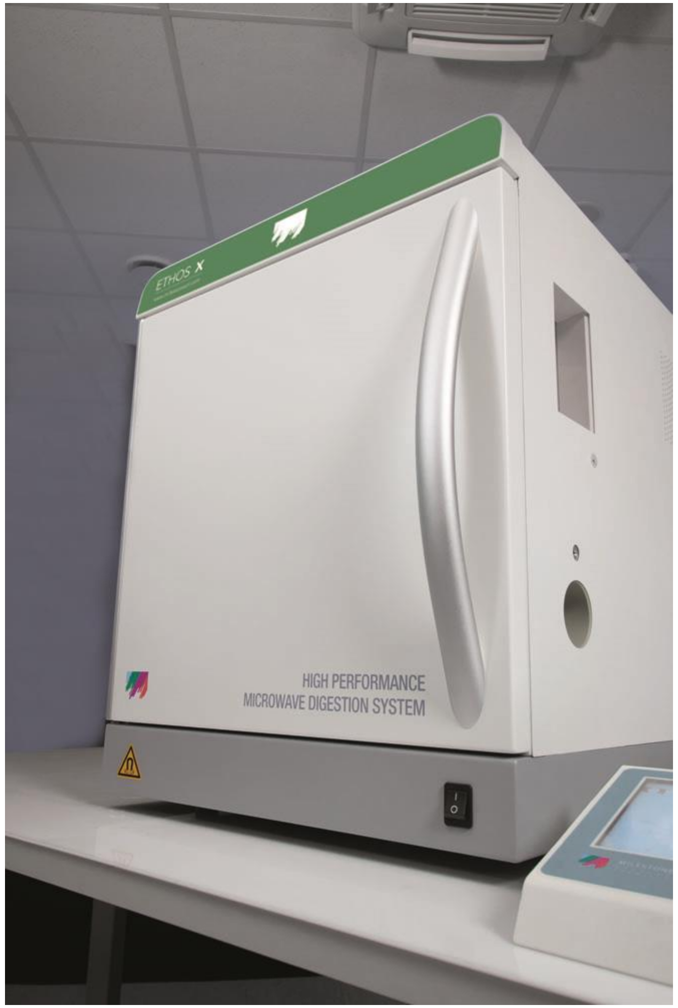
ETHOS X FEATURE

- Stainless-steel hardware construction
- Robust and rugged design
- Door block during the run to avoid handling in hot temperature
- Constant fume ventilation during analysis
- Contactless temperature control, easyTEMP



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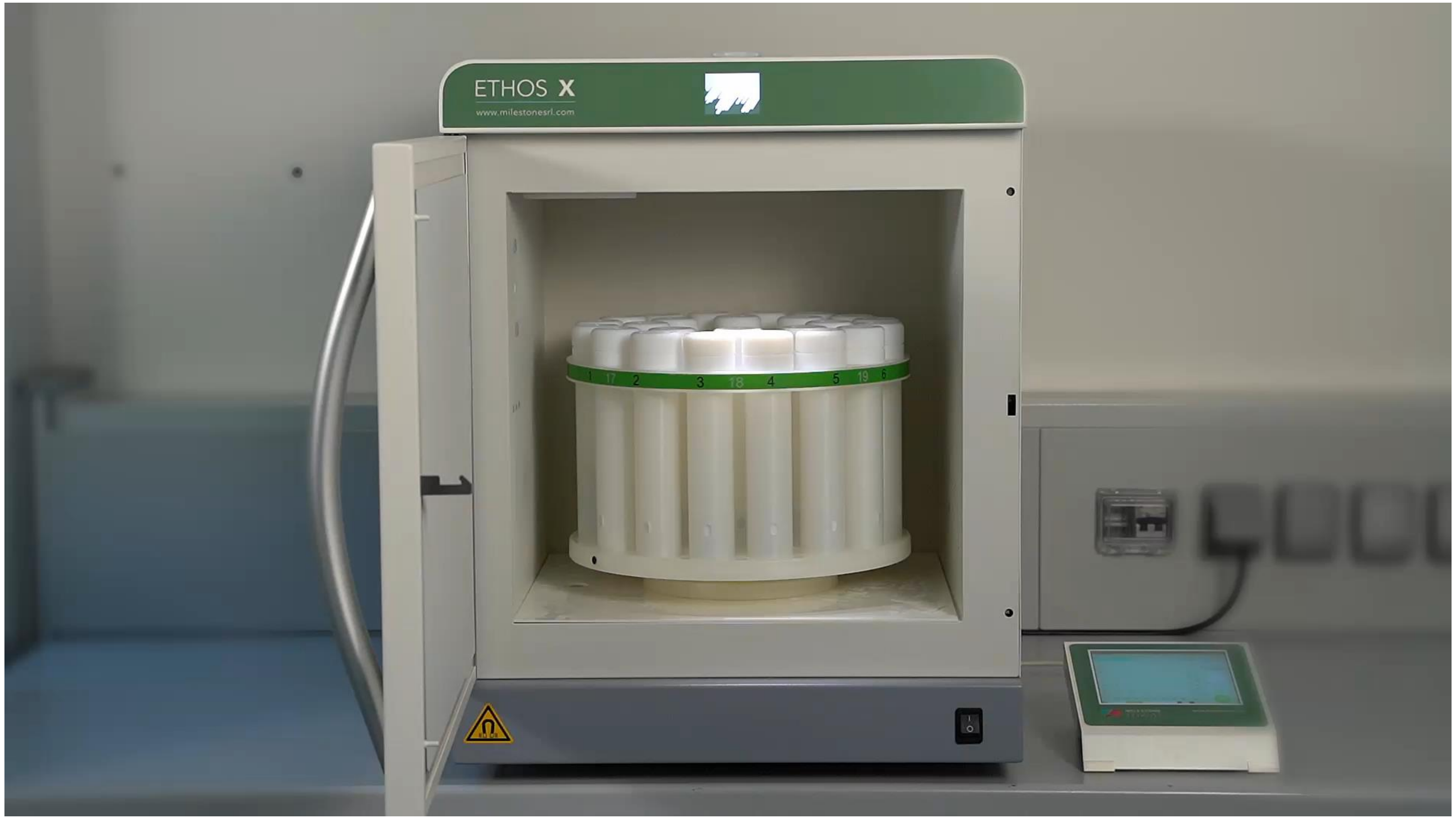
TEMPERATURE CONTROLLED IN ALL VESSELS

EASYTEMP



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THE fastEX-24ET ROTOR

- 24 positions (145mL vessels)
- 100 ml disposable glass vials
- PTFE caps and covers
- Unique vessel design: Weflon ring (modify PTFE) to ensures heat of any solvent mixtures (100 % non-polar)

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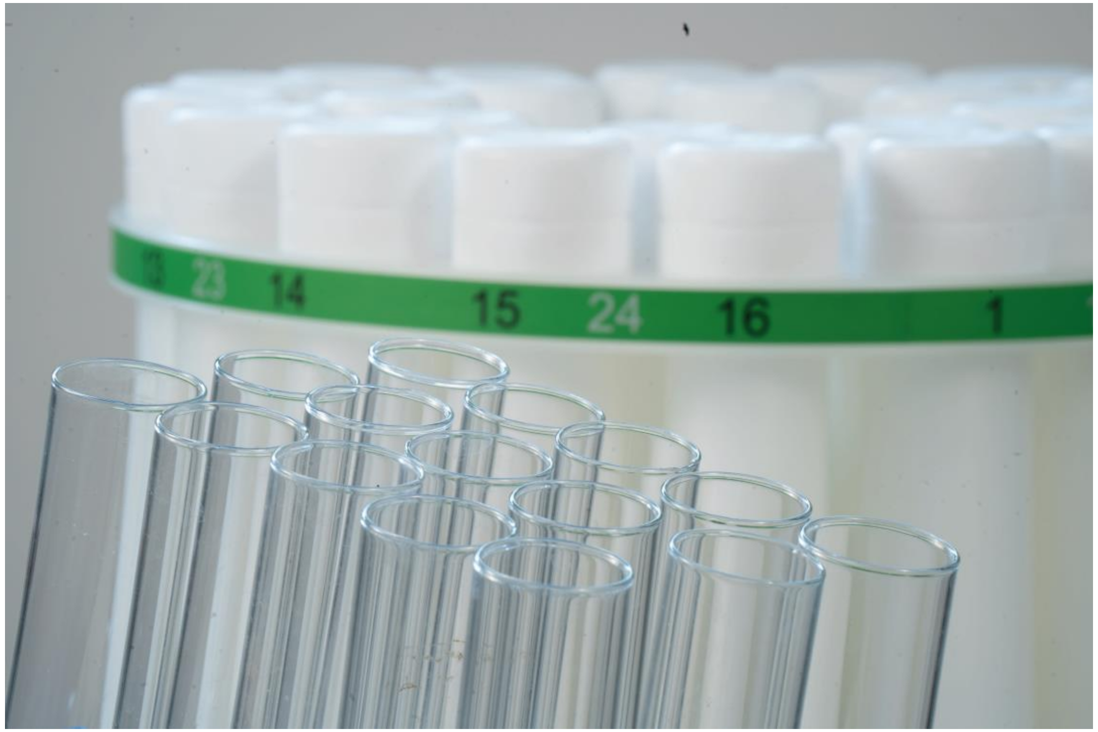
HIGH THROUGHPUT – DISPOSABLE GLASS VIAL

- No cleaning
- No memory effect
- Low running cost
- Large volume 100 ml
- Large volume allow extraction of large sample amount, up to 30g



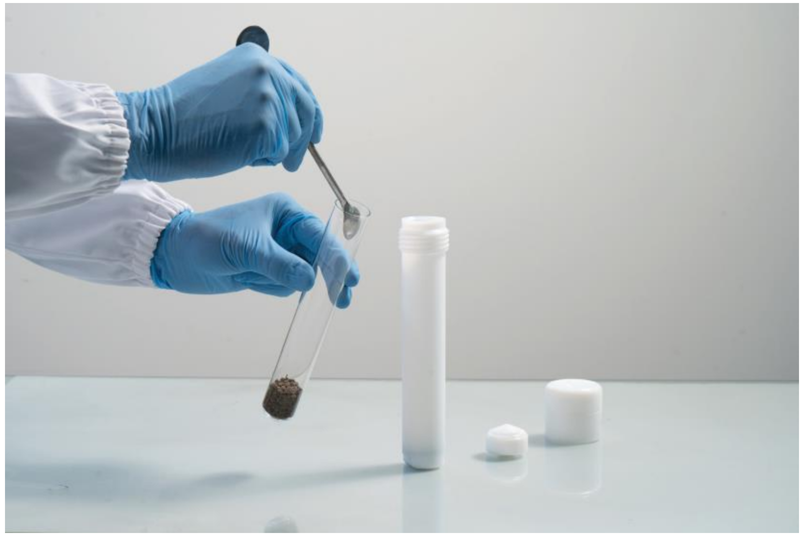
LOWER SOLVENT CONSUMPTION

Operating commonly with 25-30 mL of solvent per sample with 10 g of sample up to 50-60 ml with 30 g of sample

OPERATIVITY

1. LOAD SAMPLE, SOLVENT AND SEAL THE VESSEL

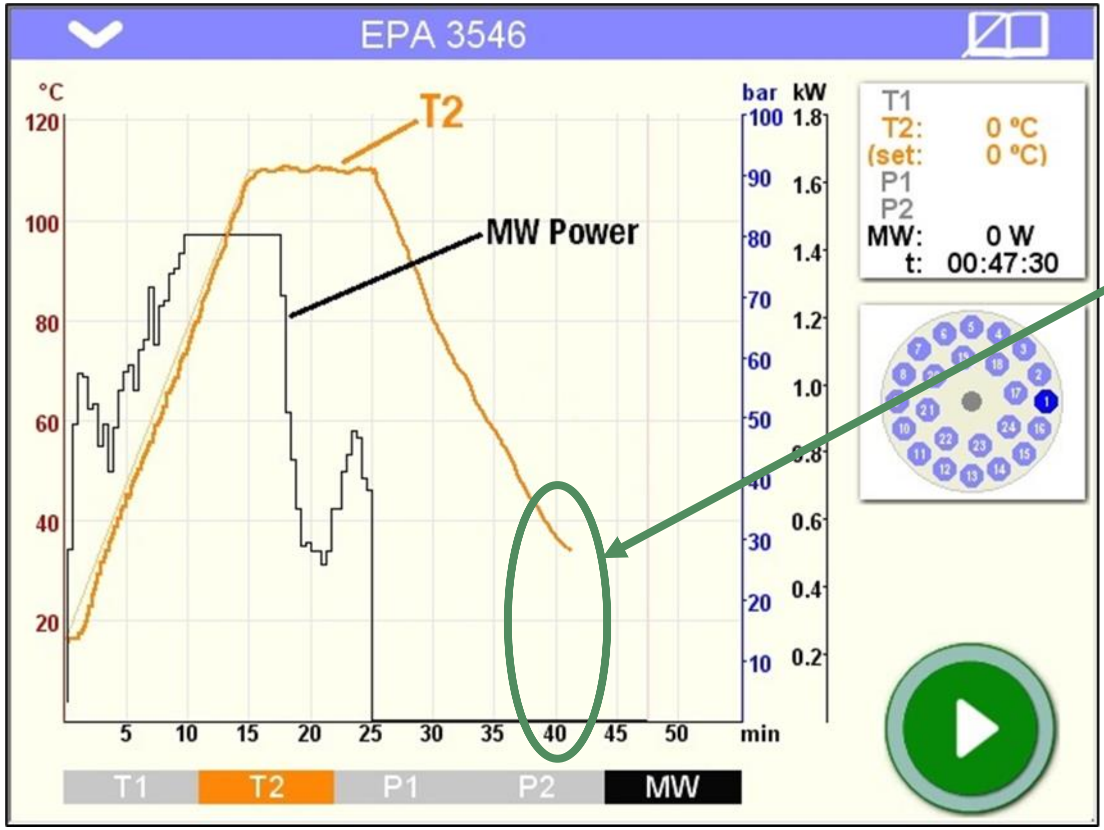
Load the sample



Add the solvent

Vessel closing

2. RUN THE MICROWAVE PROGRAM



Microwave program: 40 min

3. VESSEL OPENING

POPs EXTRACTION

RECOVERY STUDIES

DIOXINS AND FURANS

SOIL BCR-529

Analyte	Certified value (µg/kg)	Ethos X (MAE) (µg/kg)	Recovery (%)	RSD (%)
2,3,7,8-TCDD	4.5±0.6	4.2	93	3.4
1,2,3,7,8-PeCDD	0.44±0.05	0.52	118	2.8
1,2,3,4,7,8-HxCDD	1.22±0.21	1.3	106	3.1
1,2,3,6,7,8-HxCDD	5.4±0.9	4.6	85	2.1
1,2,3,7,8,9-HxCDD	3.0±0.4	2.5	83	1.9
2,3,7,8-TCDF	0.078±0.013	0.075	96	2.7
1,2,3,7,8-PeCDF	0.145±0.028	0.116	80	3.5
2,3,4,7,8-PeCDF	0.36±0.07	0.33	91	2.6
1,2,3,4,7,8-HxCDF	3.4±0.5	3.4	100	1.9
1,2,3,6,7,8-HxCDF	1.09±0.15	1.08	99	3.8
1,2,3,7,8,9-HxCDF	0.022±0.010	0.018	82	3.6
2,3,4,6,7,8-HxCDF	0.37±0.05	0.45	122	2.2

PCDD and PCDF recovery from sandy soil standard reference material BCR-529 (2g).

NO CARRYOVER EFFECT

- Changed disposable glass vial without cleaning step of the system
- No contamination of the vessels was noticed
- No cleaning step required

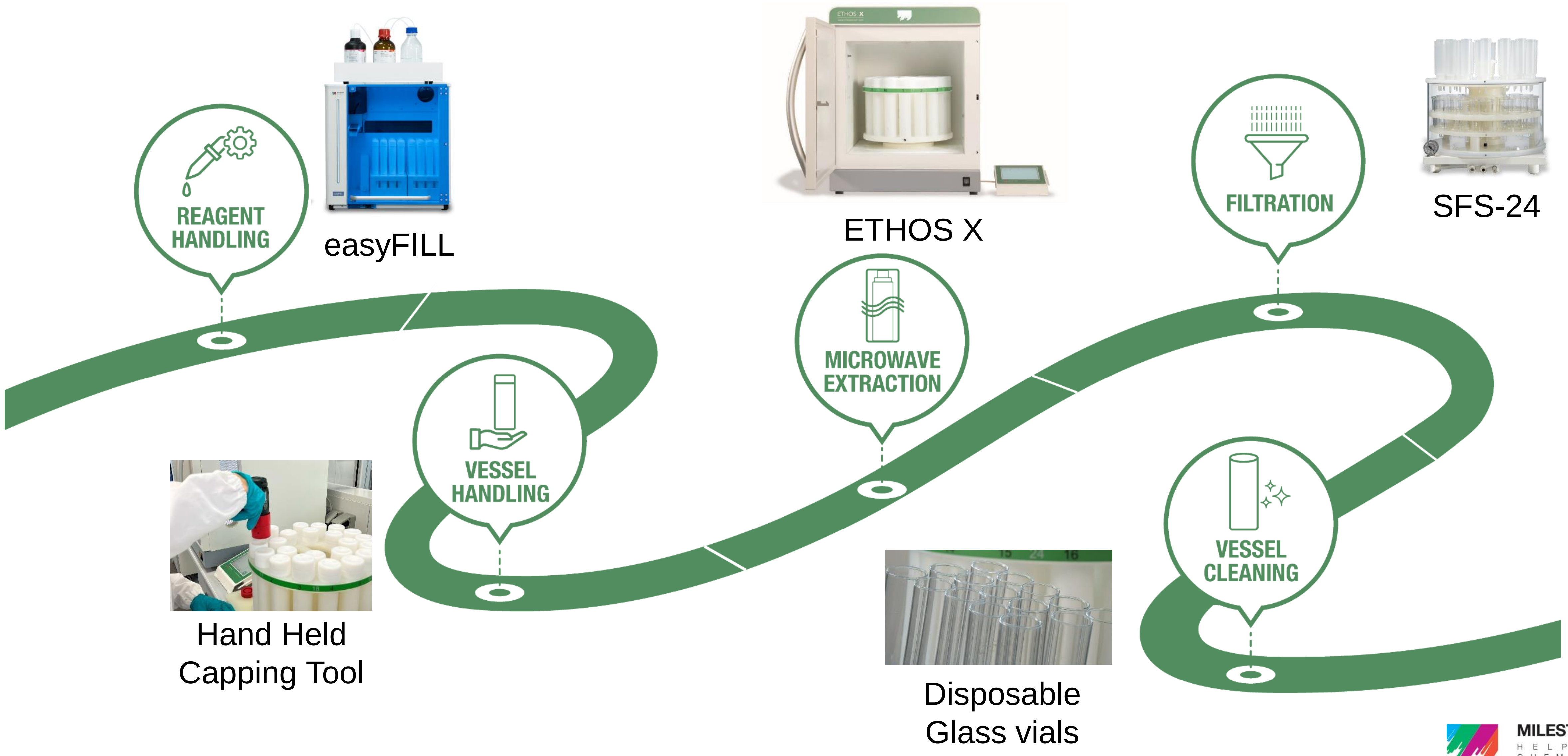
Analyte	Blank value (µg/kg))
2,3,7,8-TCDD	<0.001
1,2,3,7,8-PeCDD	<0.001
1,2,3,4,7,8-HxCDD	<0.001
1,2,3,6,7,8-HxCDD	<0.001
1,2,3,7,8,9-HxCDD	<0.001
2,3,7,8-TCDF	<0.001
1,2,3,7,8-PeCDF	<0.001
2,3,4,7,8-PeCDF	<0.001
1,2,3,4,7,8-HxCDF	<0.001
1,2,3,6,7,8-HxCDF	<0.001

APPLICATION REPORTS AVAILABLE

- TPH- Total petroleum hydrocarbons
- PAH - Polynuclear aromatic hydrocarbons
- PCB - Polychlorinated biphenyls
- Phthalate esters
- Pesticides
- Dioxins and Furans
- PBDE – Polybrominated diphenil ethers
- Phenols

More class of compound in app notes

SAMPLE PREP WORKFLOW



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ADVANTAGES OF ETHOS X FOR POPs

Fast Extraction

40 min per 24 samples

No Cleaning

Inexpensive disposable vial

Reduced Cost per Sample

With inexpensive consumables and lower reagent volume

Less operator time

Simplified handling and no cleaning

Full control of the process

Direct Contactless Temperature control in all vessels

Fast Implementation

Compliant with Official method and ease of use: EPA 3546

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TOTAL FAT DETERMINATION

WHY FAT PARAMETER IS IMPORTANT?

- Nutritional information reported nearly on all commercial food
- Quality control of feed to dose expensive components
- Nutritional information to inform the customers to choose healthy diet
- Quality control of raw materials
- Auxiliary step for subsequent analyses:
    - Lipids evaluation,
    - Contaminants,
    - Etc…

Nutrition Facts

Serving Size	**100g**
Amount Per Serving	
Calories	**420**
	% Daily Value*
Total Fat 20 g	**5%**
Saturated Fat 1 g	10%
Trans Fat 15 g	70%
Polyunsaturated Fat 0 g	0%
Monounsaturated Fat 0 g	0%
Cholesterol 15 mg	**2%**
Sodium 3 mg	**0%**
Total Carbohydrate 50 g	**3%**
Dietary Fiber 8 g	12%
Total Sugars 25 g	34%
Includes Added Sugars 0.1 g	0.1%
Other Carbohydrate 0.1 g	0.2%
Protein 50 g	**20%**
Vitamin A	75%
Vitamin C	50%
Vitamin D	15%
Vitamin E	65%
Vitamin K	70%
Thiamin (Vitamin B1)	5%
Riboflavin (Vitamin B2)	10%
Niacin (Vitamin B3)	15%
Vitamin B6	25%
Folate (Vitamin B9)	90%
Vitamin B12	90%
Biotin (Vitamin B7)	45%
Pantothenic Acid (Vitamin B5)	20%
Choline	5%
Calcium	80%

* The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. Percentages are based on a 2000 calories diet

US FDA
http://www.fda.gov/nutritioneducation

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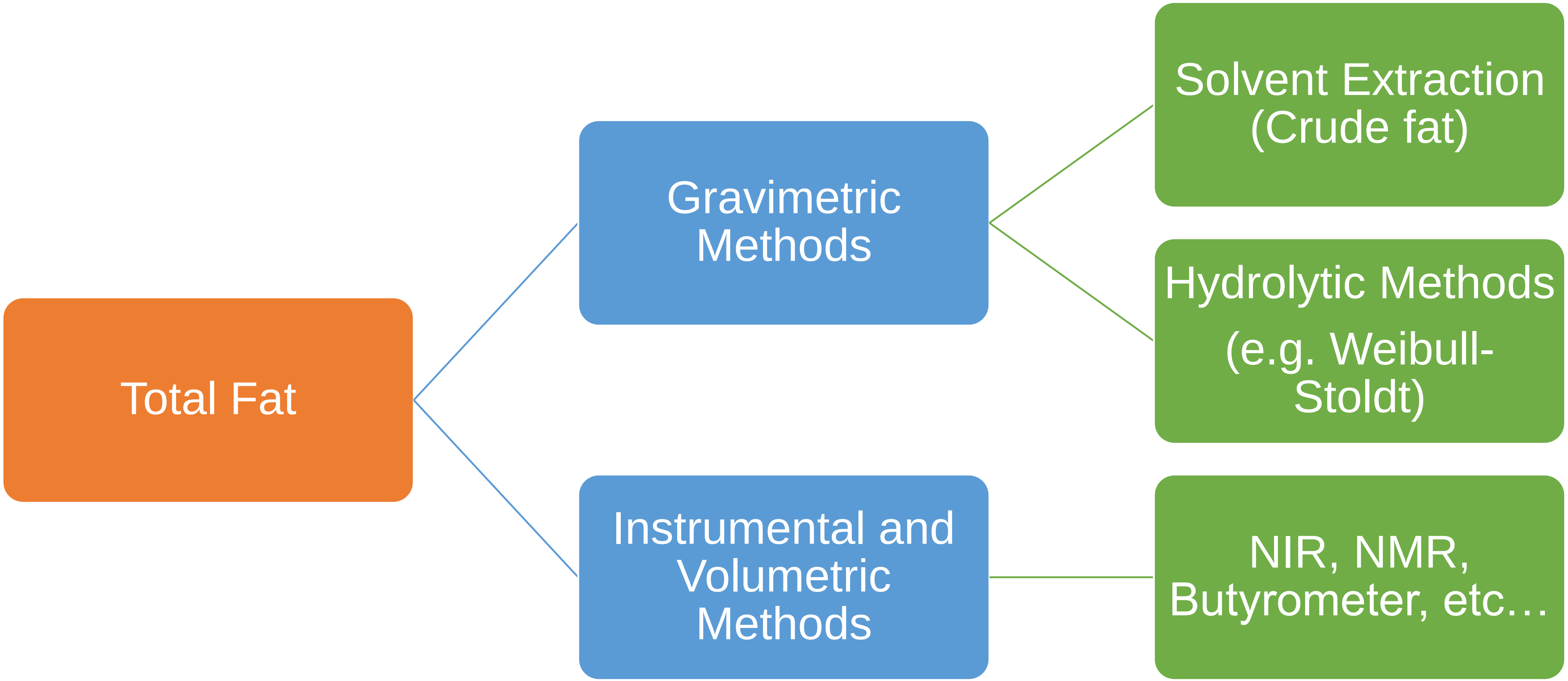
HOW TOTAL FAT IS DETERMINED?



TRADITIONAL APPARATUS FOR TOTAL FAT

+

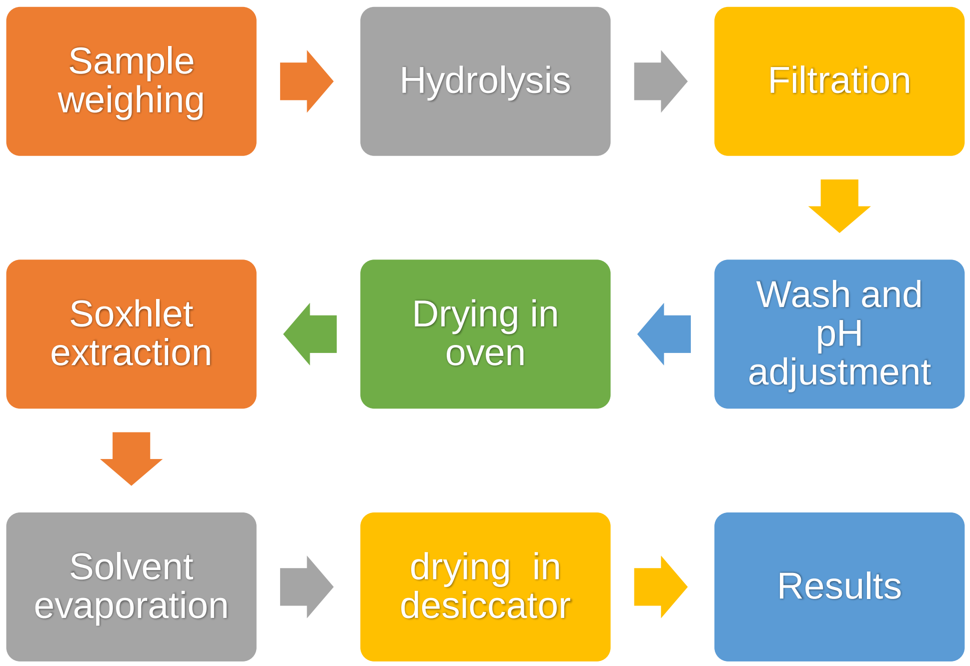
OR

acid hydrolysis bath

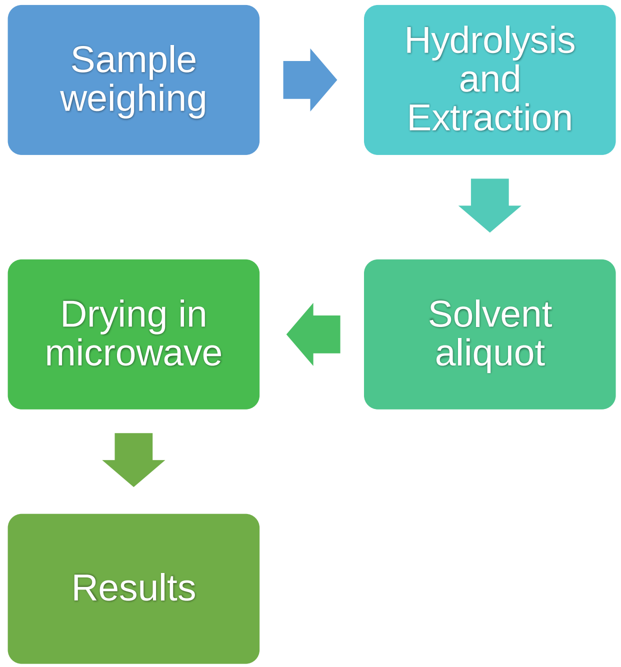
Traditional Soxhlet apparatus

Automatic Soxhlet apparatus

CONVENTIONAL VS ETHOS X - PROCESS



CONVENTIONAL
WEIBULL-STOLDT METHOD
# of steps: 9



ETHOS X
# of steps: 5

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CONVENTIONAL VS ETHOS X - TIMING

Conventional Approach

Weighing & Sample prep

Hydrolysis

Drying

Extraction

Evaporation

Work up

Weighing

Calculation

Microwave Approach

Weighing

Hydrolysis & Extraction

Prep for evaporation

Evaporation

Weighing & Calculation

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TOTAL FAT - MICROWAVE HARDWARE

ETHOS X FOR FAT HARDWARE

Ethos X

SR-15
Rotor

RAR-15
Rotor

Analytical
balance

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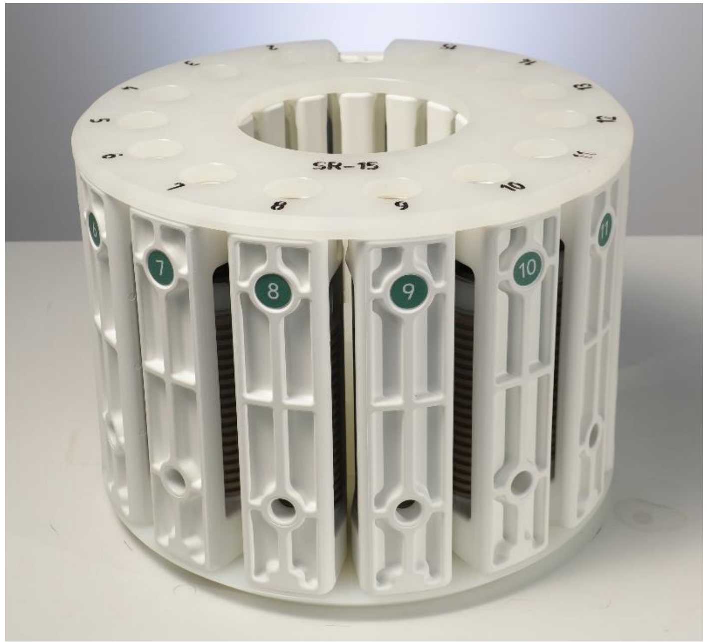
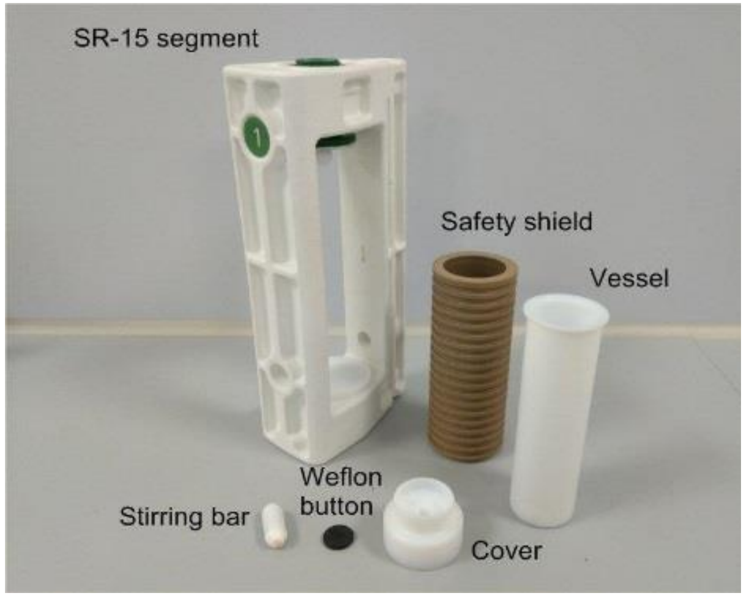
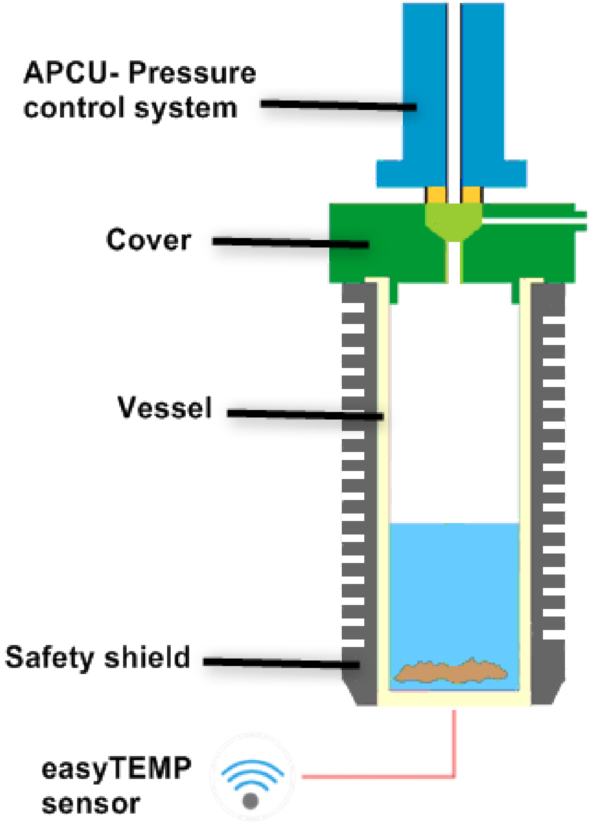
SR-15 ROTOR TECHNOLOGY







Rotor	Min volume	Max volume	Max working temperature	Max working pressure
SR-15 eT	5 ml	100 ml	180°C	35 bar

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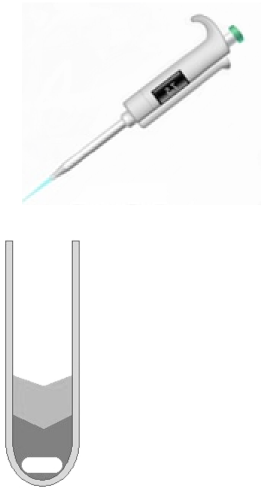
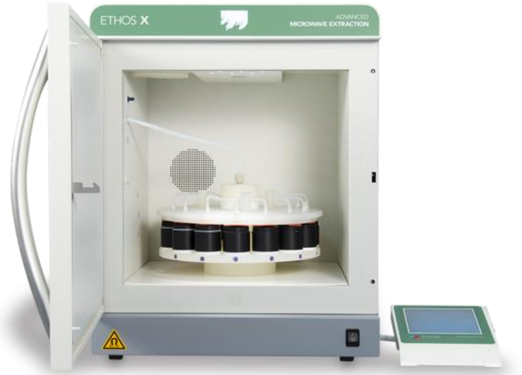
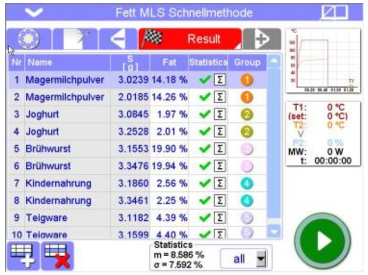
TOTAL FAT - PROCEDURE

TOTAL FAT PROCEDURE

Sample weighing → Hydrolysis and Extraction → Solvent aliquot → Drying in RAR15 → Results







For one sample: 10 mL H_2SO_4 25% for hydrolysis; 25 mL cyclohexane for extraction

TOTAL FAT

RECOVERY STUDIES

CERTIFIED REFERENCE MATERIALS

Sample Type	Sample ID	Certified Value		ETHOS X Results		
		Total Fat (%)	Uncertainty (%)	Total Fat (%)	n	Std. Dev. (%)
Whole milk powder	BCR-380R	26.95	± 0.16	26.3	11	0.23
Wheat Flour	ERM-BC382	1.39	± 0.17	1.41	12	0.2
Lyophilized Pork muscle	ERM-BB384	8.99	± 0.2	8.63	6	0.21
Condensed Milk	TET036RM	0.33	± 0.07	0.29	12	0.06
Dairy feed	BCR-708	6.5	± 0.8	6.32	9	0.28

Sample Type	Sample ID	Certified Value		ETHOS X Results		
		Total Fat (%)	Acceptability [Range for IzI≤2] (%)	Total Fat (%)	n	Std. Dev. (%)
Porridge Oats	T2477QC	7.82	7.36 – 8.28	8.03	12	0.187
Butter	T25160QC	81.37	80.83 – 81.91	81.38	6	1.28
Fish Paste	T25163QC	4.43	3.77 – 5.10	4.4	6	0.07
Chocolate	T25166QC	34.85	33.52 – 36.17	35.74	12	0.67
Fat Spread	T14190QC	66.47	64.8 - 68.1	68.0	12	0.48

CASE STUDY – EUROFINS FRANCE

CUSTOMER

- Eurofins Scientific is the recognized leader in food and pharmaceutical product testing.
- Site in Nantes is the headquarter of Eurofins, main food parameters laboratory (nutriscience)

CHALLENGE

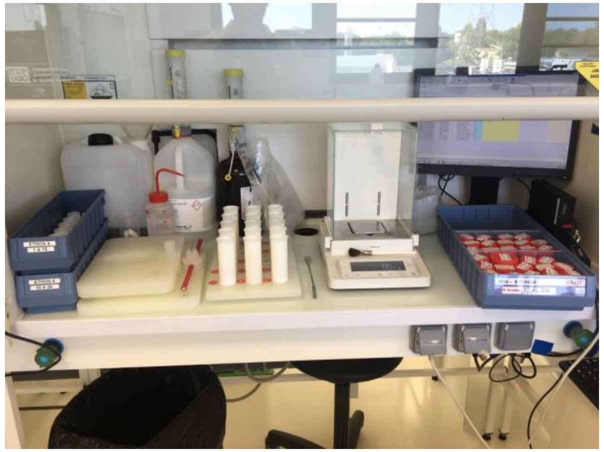
- Increase their productivity. Goal of processing **450 samples** received **per day** and to produce **results faster**
- The **current** sequential technology was **time consuming** and **not able to provide reproducible results** on samples with very low-fat content.

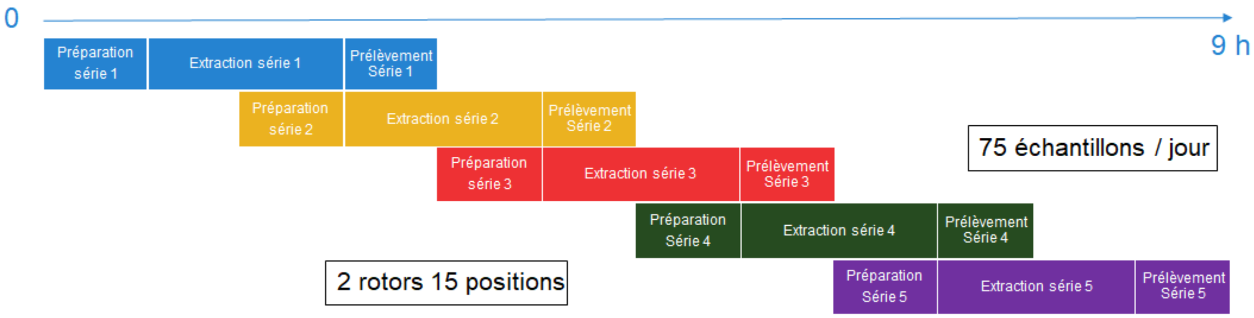
.

EUROFINS FRANCE ACTUAL WORKFLOW

- 7 Ethos X + 10 x SR-15 rotors
- 450 samples a day wide range of matrices
- Profitability : x 18
- Lower cost and turnaround time
  - Results delivered to customer within a day
- Over 85% of ETHOS X method acceptance from customers.

They are now investing in technology across Europe





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CASE STUDY – DE HEUS

CUSTOMER

- **De Heus** Animal Nutrition is an international producer of a **complete range of compound feed**

CHALLENGE

- **Reduce analysis time**
- produce more **accurate and reliable results** on several raw materials and feed matrices

Dr. Rowan Bosch, Laboratory Manager, De Heus Animal Nutrition

FOOD PROCESSORS – CASE STUDY

"The first reason we chose ETHOS X was the need for faster results. If I were to carry out 45 samples in the traditional way, it would take a full week, including preparation. Now I can quickly manage 30 samples every day."

Rowan Bosch, Laboratory Manager, De Heus Animal Nutrition

De Heus is investing in ETHOS X and it has already installed it in Vietnam and Poland facility

OTHER FOOD APPLICATION

APPLICATION REPORT
MW assisted sample preparation for FAME determination

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VALIDATION AGAINST AOCS PROTOCOLS OF MICROWAVE-ASSISTED SAMPLE PREPARATION FOR FAMEs ANALYSIS

Fatty Acid Methyl Ester (FAME) analysis is commonly used to calculate saturated, monounsaturated, and polyunsaturated fat contents in both food and feed industries and related third-party quality control laboratories. A great number of these laboratories follow various AOCS methods, which rely on the use of standard and outdated techniques, usually employing hazardous reagents. FAME protocols based on the Milestone's ETHOS X Microwave-assisted technology are a solid alternative to the conventional technique. This work aims to provide a demonstration of the reliability of this microwave-assisted method against the conventional AOCS procedures.

INTRODUCTION
In the U.S. and EU, fat determination in food is subjected to regulatory standards that ensure accurate labeling and quality control. In the U.S., FDA and USDA are the organizations in charge of the fat analysis legislation [1], specifying methods based on food type, such as total fat extraction. Similarly, EU follows standardized protocols under EFSA and has adopted the subject under Regulation (EU) No 1169/2011 [2].
One of the most widely used techniques for fat characterization is the conversion of fat to fatty acid methyl esters (FAMEs), which allows detailed profiling of the fatty acid composition through gas chromatography (GC). This process, subject of AOCS methods (AOCS Ce 2b-11 and 2c-11), involves lipid extraction followed by transesterification to form FAMEs, enabling precise measurement of individual fatty acids. Despite its accuracy, traditional FAME analysis methods involve lengthy and solvent-intensive steps, which are not efficient and not the best choice for the environment.

Recent advancements have drawn the attention to microwave-assisted extraction (MAE) as a potential improvement over the conventional method. In fact, MAE reduces both solvent usage and extraction time by applying microwave energy to accelerate lipid extraction and transesterification. This technique has been shown to produce comparable or superior accuracy to standard methods and has the added benefit of lowering operational costs.
The aim of the work presented in this report is the comparison between different ways to get FAMEs profile in different food matrices.
Specifically, we compared two AOCS methods for FAMEs preparation (alkaline and acid conditions) with MAE derivatization conducted on fat extracted with or without hydrolysis, and the innovative all-in-one-stage MAED technique.
Overall, seven different methods on six different food matrices were evaluated, followed by a common analysis by GC (GC x GC)- FID.

Not only Total Fat:

- Free Fat
- FAMEs derivatization (university of Liege collaboration
- Saponification for sterols and DAK
- Protein hydrolysis
- etc

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ADVANTAGES OF ETHOS X FOR FAT

- High productivity
- Universal for any matrix, food & feed
- Automatic and Easy-to-use
- Compact, save space in the lab
- Reduced footprint of the laboratory
- Limited reagent consumpion-Green approach

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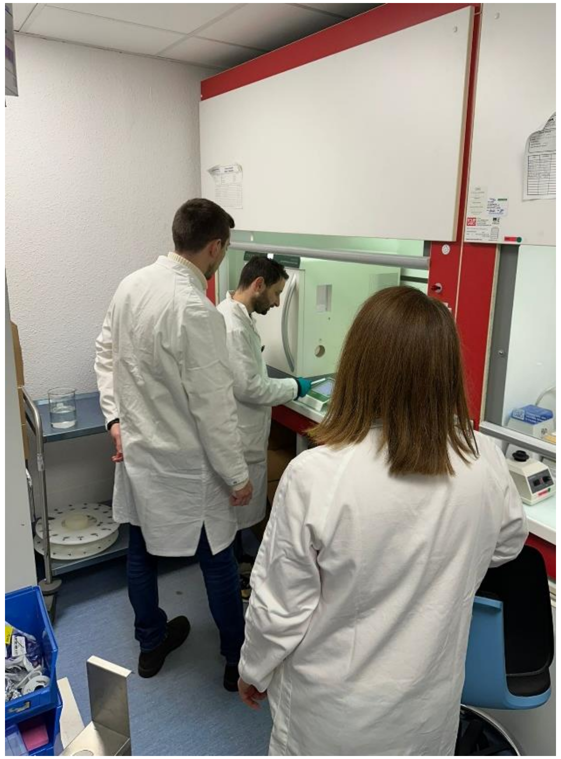
ETHOS FLEXIBILITY

DEMO WEEK



We are organizing a demo week in Finland in May

We will run your samples together

THANK YOU