



# 19ENG09 BIOFMET

New metrological methods for biofuel materials analysis  
(June 2020-May 2023)



The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

## Production and Certification of BIOFMET Project Reference Materials

**2nd BIOFMET Stakeholders' Workshop  
Workshop on metrology for biofuel industry**

**29 March 2023  
Alper İşleyen, TÜBİTAK UME**

Partners:



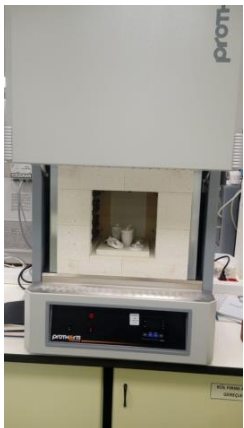
Collaborators:



DANISH  
TECHNOLOGICAL  
INSTITUTE



- Reference Materials: Usage Areas and Production Steps
- Planning for Production of Biofuel Reference Materials
- Processing of Solid and Liquid Biofuel Reference Materials
- Homogeneity and Stability Assessments
- Characterization Strategies, Value and Uncertainty Assignment
- Comparison of Two Moisture Analysis Methods with Candidate Solid RMs
- Improvement of Ash Measurements



## Reference Material (RM)

Material, sufficiently **homogeneous** and **stable** with respect to one or more specified **properties**, which has been established to be fit for its intended use in a measurement process.

**Properties** can be quantitative or qualitative, e.g. identity of substances or species.

## Certified Reference Material (CRM)

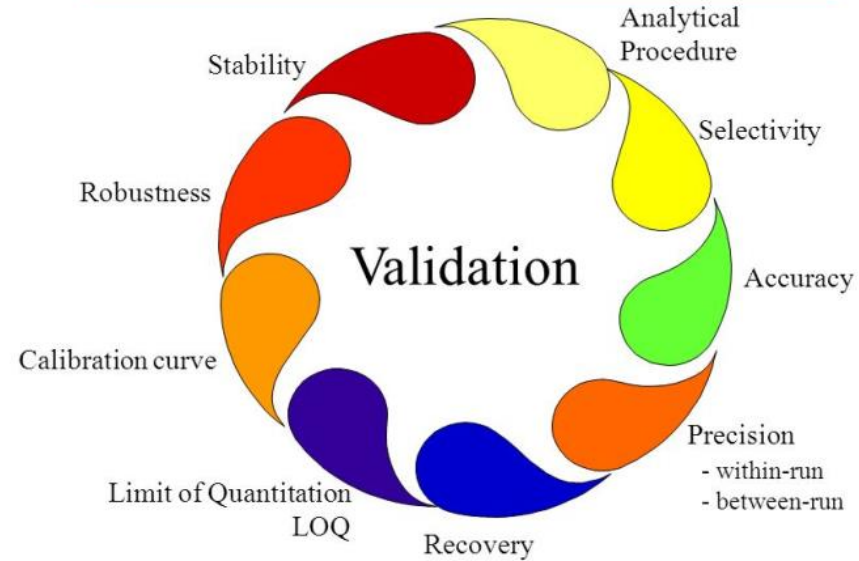
Reference material characterized by a **metrologically valid procedure** for one or more specified properties, accompanied by a RM **certificate** that provides the **value** of the **specified property**, its associated **uncertainty**, and a statement of **metrological traceability**.



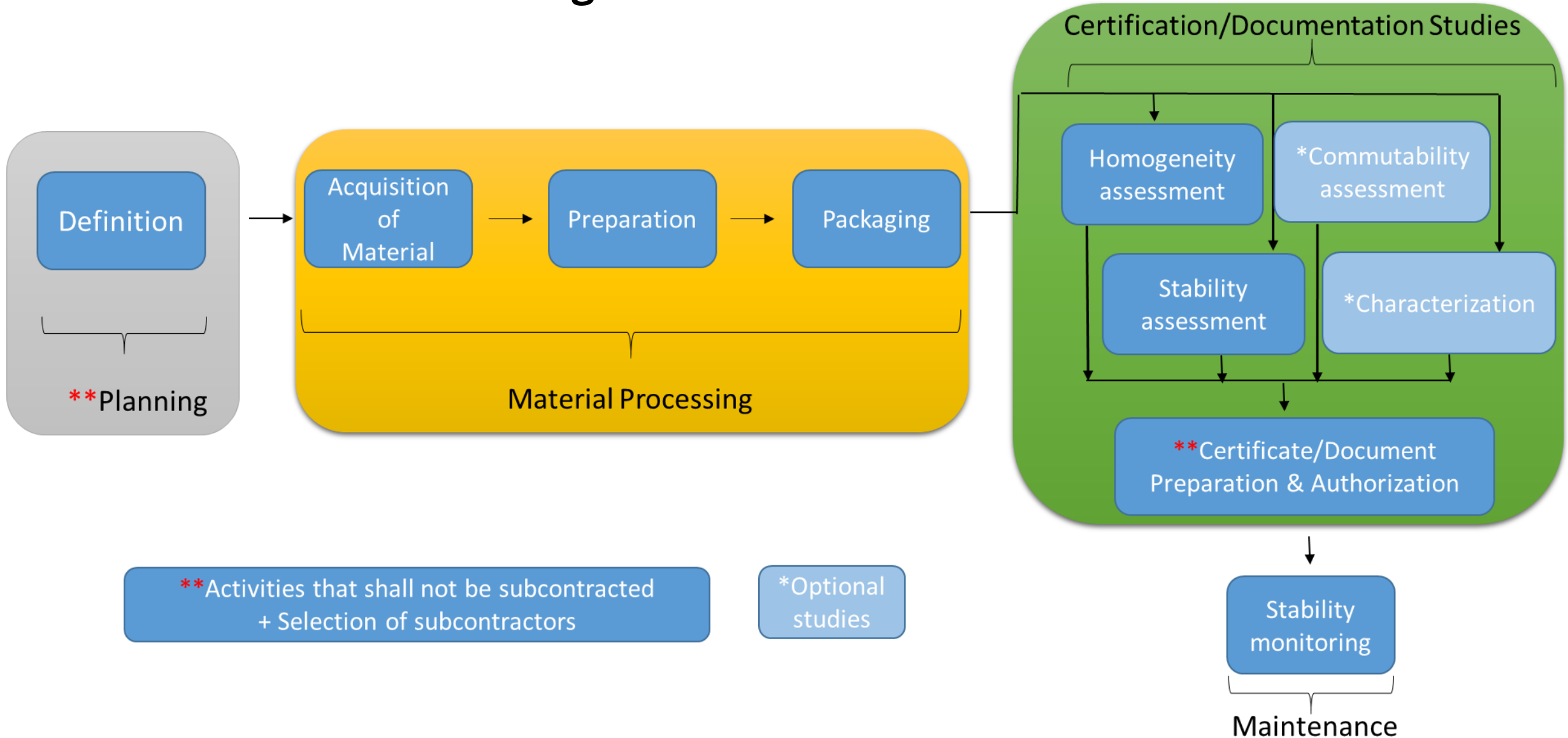
- Method development
- Method validation
  - Evaluation of trueness
  - Estimation of uncertainty
- Calibration
- Method performance charts
  - Within Lab Quality Control ('charts')
  - Evaluation of analyst or instrument performance
- Proficiency Tests
  - Validation of Proficiency (external comparison)
- Assigning value to other materials

Metrological Traceability

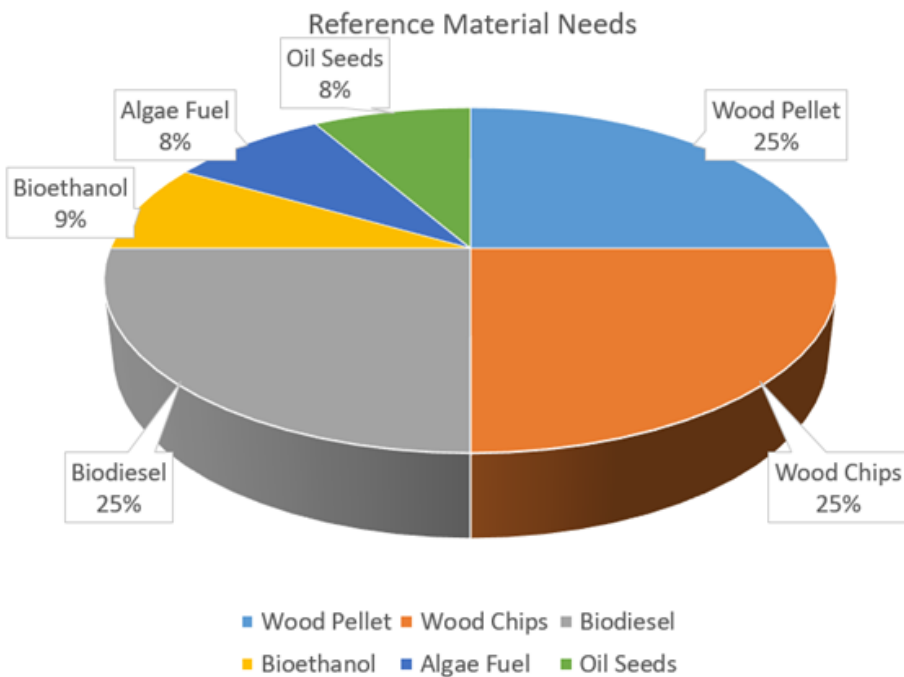
## Analytical method validation



# Main Steps in Producing Reference Materials according to ISO 17034 Standard



A questionnaire and literature survey (e.g. present CRMs, relevant standard methods) were initiated to collect information about reference material needs for solid and liquid biofuel analysis.



PARAMETERS OF INTEREST BY SURVEY PARTICIPANTS			
Wood Pellet	Wood Chip	Biodiesel	Bioethanol
Gross Calorific Value	Calorific Value	ISO EN 14214 Parameters	ISO EN 15376 Parameters
Net Calorific Value	Ash	Fame Content	Ethanol + Higher Saturated Alcohols content
Ash	Moisture	Density at 15 C	Higher saturated (C3-C5) mono-alcohols content
Volatile Matter	C	Viscosity at 40 C	Methanol content
S	H	Flash Point	Water content
F	N	Cetane Number	Total Acidity
Cl	Cl	Copper Strip Corrosion (3 h at 50 C)	Electrical conductivity
H	S	Oxidation Stability (at 110C)	Inorganic chloride content
N	Heavy Metals	Acid Value	Sulfate content
O		Iodine Value	Copper content
Fixed Carbon		Linoleic acid methyl ester	Phosphorus content
Metal Oxides in Ash		Polyunsaturated (>=4 double bonds) methyl esters	Involatile material content
		Methanol content	Sulfur content
		Monoglyceride content	
		Diglyceride content	
		Triglyceride content	
		Free Glycerol	
		Total Glycerol	
		Water content	
		Total Contamination	
		Sulfated Ash content	
		Sulfur content	
		Group I Metals (Na +K)	
		Group II Metals (Ca + Mg)	
		Phosphorus Content	



## UME BIOFMET CRM 01 BIODIESEL



500 mL, Amber Glass

### Targetted Parameters

#### Parameters in EN 14214

**Calorific Value**  
**Density at 15 °C**  
**Viscosity at 40 °C**  
 Methanol content  
 Monoglyceride content  
 Diglyceride content  
 Triglyceride content  
 Free glycerol  
 Total glycerol  
 Water content  
**Sulfur, S\***  
**Group I metals (Na+K)\***  
**Group II metals (Ca+Mg)\***  
**Phosphorus, P\***

## UME BIOFMET CRM 02 WOOD PELLET POWDER



50 g, Amber Glass

### Targetted Parameters

#### Parameters in EN 17225-2

**Calorific Value**  
**Moisture**  
**Ash**  
 Nitrogen, N  
**Sulfur, S**  
 Chlorine, Cl  
**Arsenic, As\***  
**Cadmium, Cd**  
**Chromium, Cr\***  
**Copper, Cu**  
**Lead, Pb\***  
**Mercury, Hg\***  
**Nickel, Ni\***  
**Zinc, Zn**

#### Parameters in EN ISO 16967

Aluminum, Al  
 Calcium, Ca  
 Iron, Fe  
 Magnesium, Mg  
 Phosphorus, P  
 Potassium, K  
 Silicon, Si  
 Sodium, Na  
 Titanium, Ti

#### Other Parameters of Interest

Carbon, C  
 Hydrogen, H  
 Manganese, Mn

## UME BIOFMET CRM 03 WOOD PELLET



100 g, Amber Glass

### Targetted Parameters

#### Parameters in EN 17225-2

**Calorific Value**  
**Moisture**



**\*Elements selected to be spiked to reach target levels in candidate CRMs**

# Processing of Biodiesel CRM Candidate



Biodiesel (B100) material  
( 70 x 5 L)  
Source: Romania  
Supplied by BRML (Romania)



0.7 µm glass fiber vacuum filtering



Combining filtered biodiesel  
in 320 L HDPE container



Spiking P, K, Na, Ca, Mg  
elements in mineral oil



Filling the 320 L container and  
circulating the content for homogenization



Bottle filling and cap closure



Labelling and test units separation  
Total: 553 units of 500 mL





Wood Pellet material  
(6 x 15 kg)  
Source: Poland  
Supplied by DTI (Denmark)



Spiking of elements to pellet  
(As, Cr, Hg, Pb, Ni)



Drying spiked pellets  
at 35 °C in hepa filtered  
air flow oven for a week



Milling of spiked & unspiked pellets  
to < 0.5 mm by cutting mill  
(2 weeks)



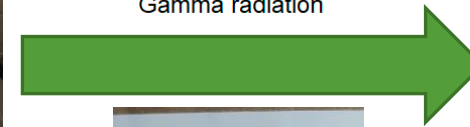
3D homogenization  
of wood powder  
(75 kg, 16 hrs)



Semiautomatic filling  
and capping



Gamma radiation



Labelling and test units separation  
Total: 1463 units of 50 g

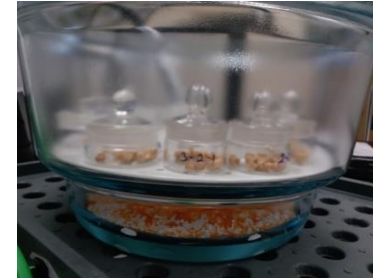
# Processing of Wood Pellet CRM Candidate



Wood Pellet material  
(4 x 15 kg)  
Source: Poland  
Supplied by DTI (Denmark)



Moisture check and  
selecting converging packages

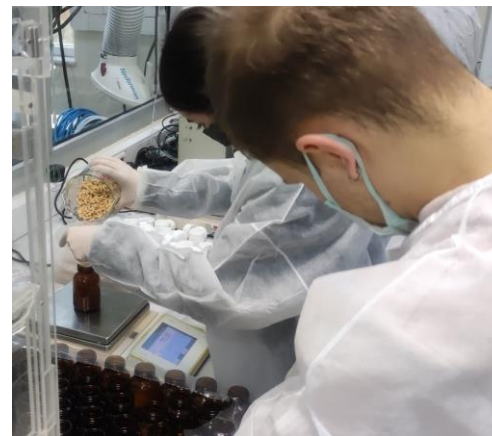


Gentle homogenization  
by transferring content between  
120 L and 60 L HDPE containers

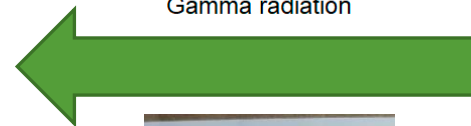


Splitting homogenized material  
into vacuum containers

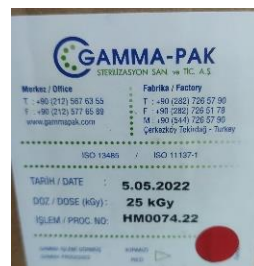
Moisture  
Check



Manual filling of pellets on a  
balance and capping



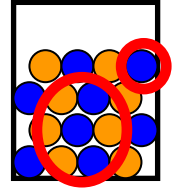
Gamma radiation



Labelling and test units separation  
Total: 571 units of 100 g

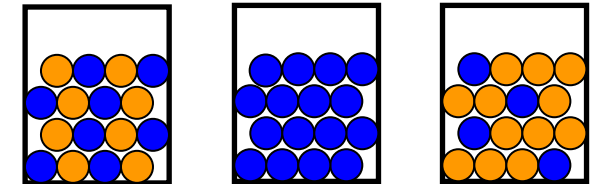
- **Within Unit Homogeneity**

- ✓ Heterogeneity can be eliminated by increasing minimum sample intake
- ✓ Important to determine the minimum amount for sample intake



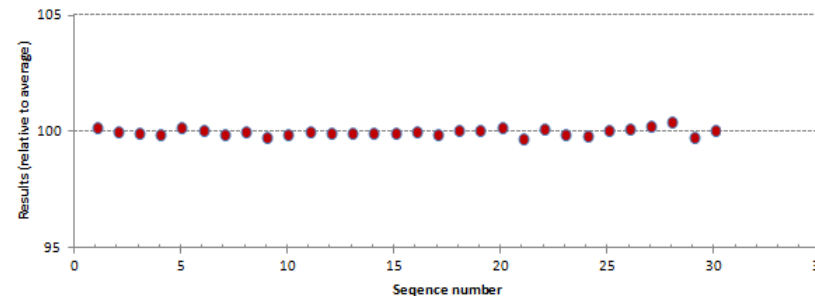
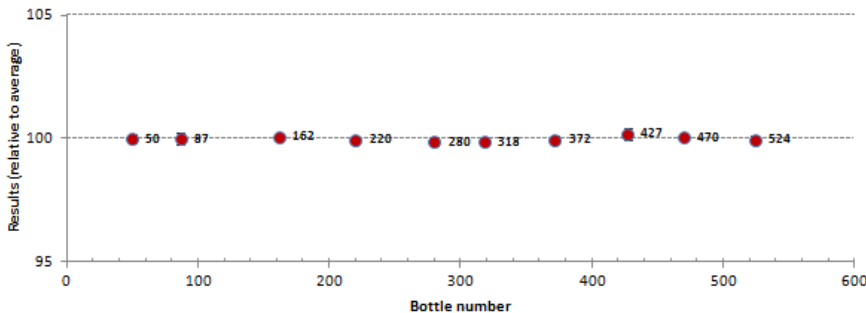
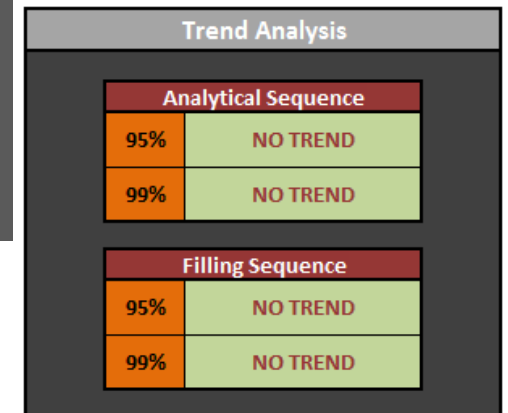
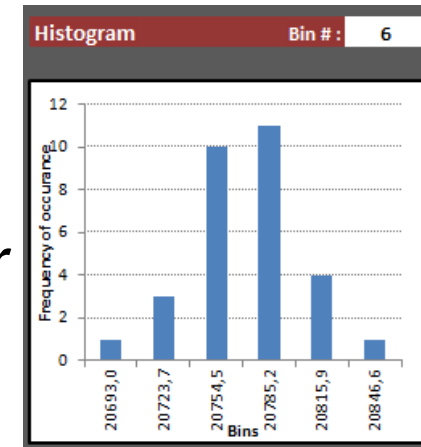
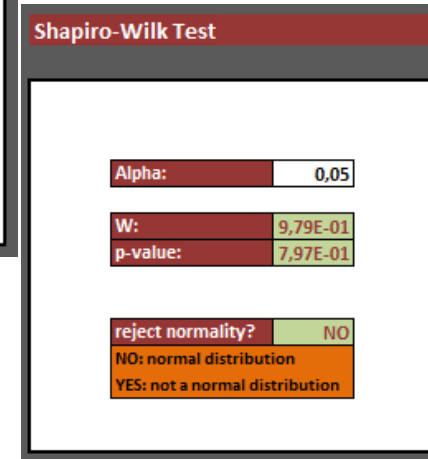
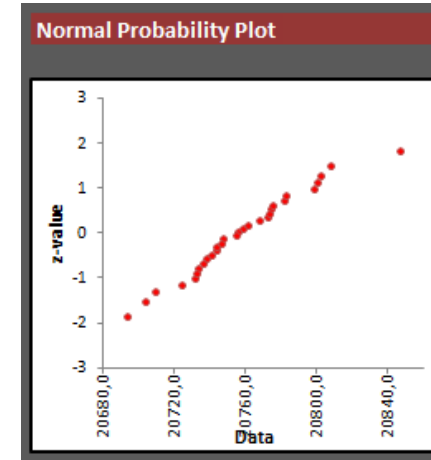
- **Between Units Homogeneity**

- ✓ Present Heterogeneity between units can not be eliminated by increasing sample intake.
- ✓ Has effect on the uncertainty of the CRM.
- ✓ Obtained data is used to calculate the uncertainty due to heterogeneity between the units.
- ✓ 10 or  $\sqrt[3]{\text{total number of units produced}}$  (whichever bigger) is tested.





- Normal distribution investigation (Normal Probability Plots and Shapiro-Wilk Test)
- Outlier Controls (Grubbs Test)
- Unimodality (Histogram)
- Trend tests for filling order and analysis order (Linest Test)





# Homogeneity -Uncertainty Calculations

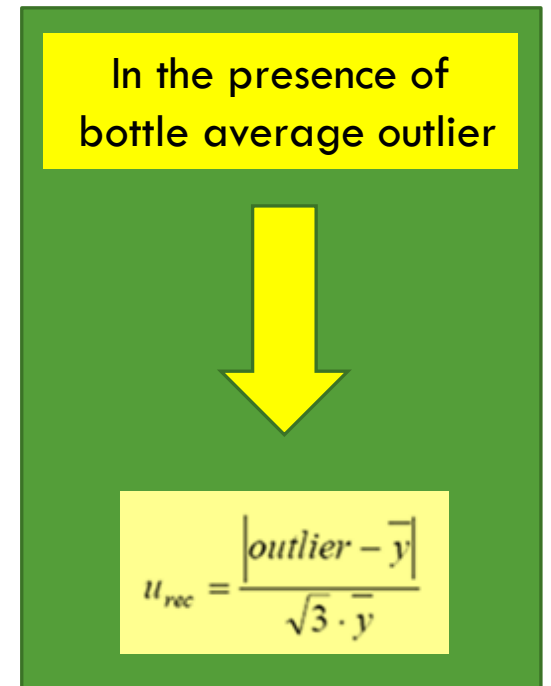
$$S_{wb,rel} = \frac{\sqrt{MS_{within}}}{\bar{y}}$$

$$S_{bb,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}}$$

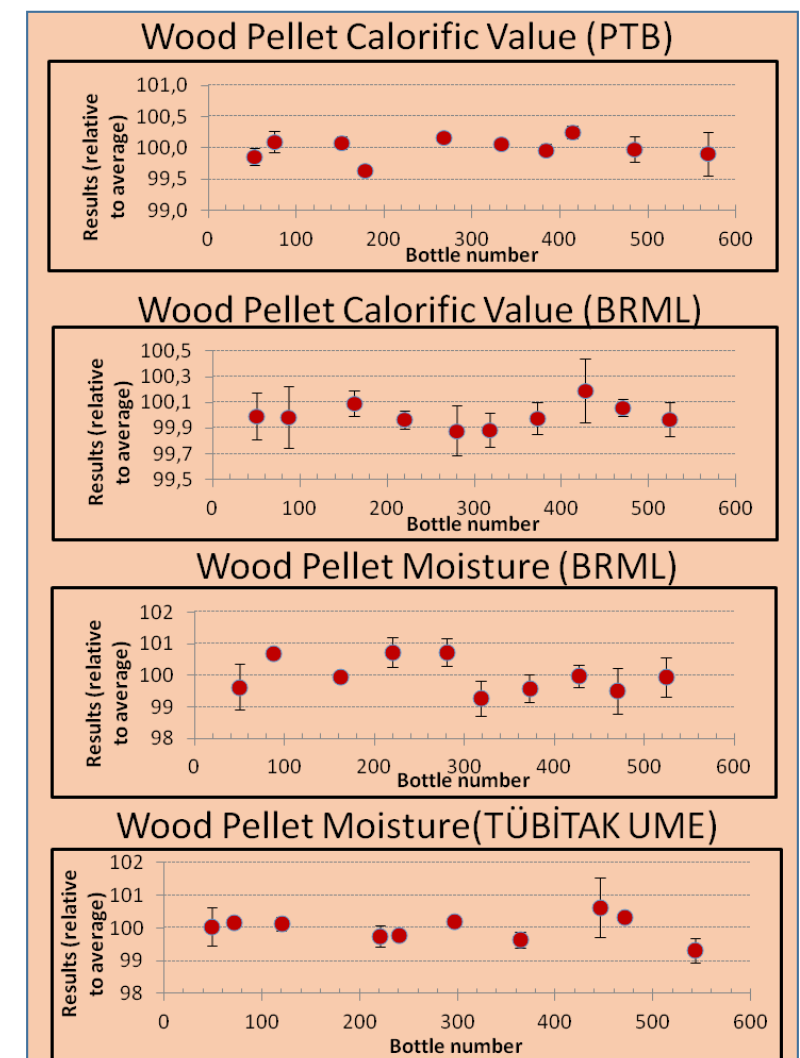
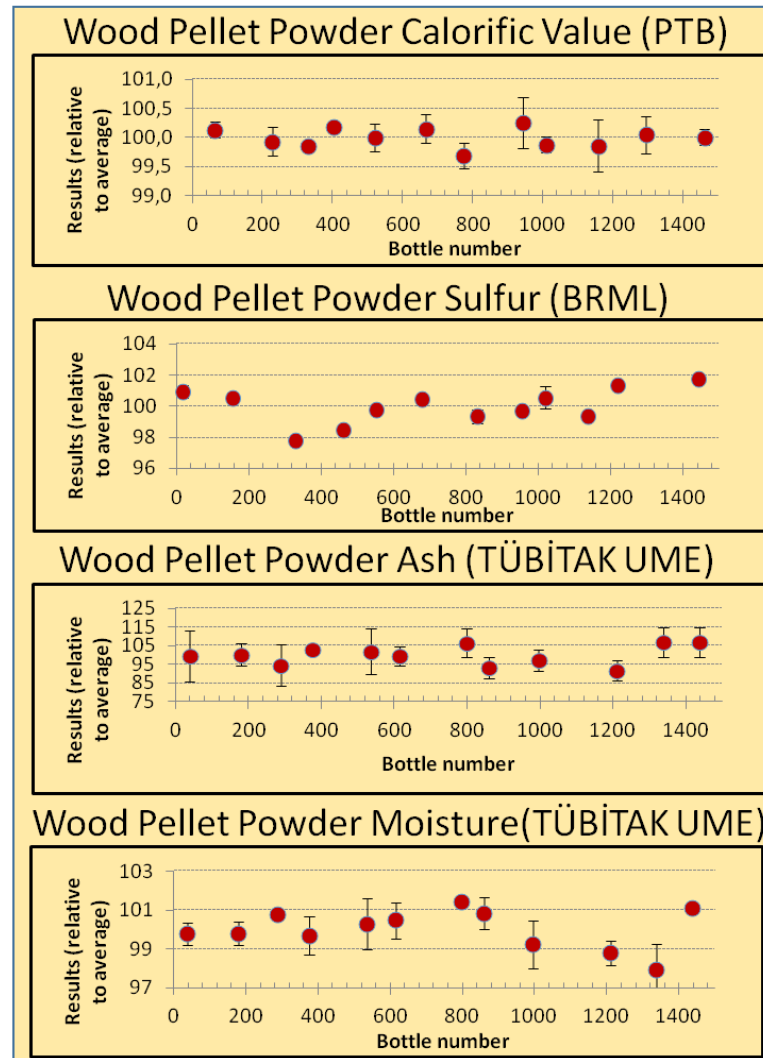
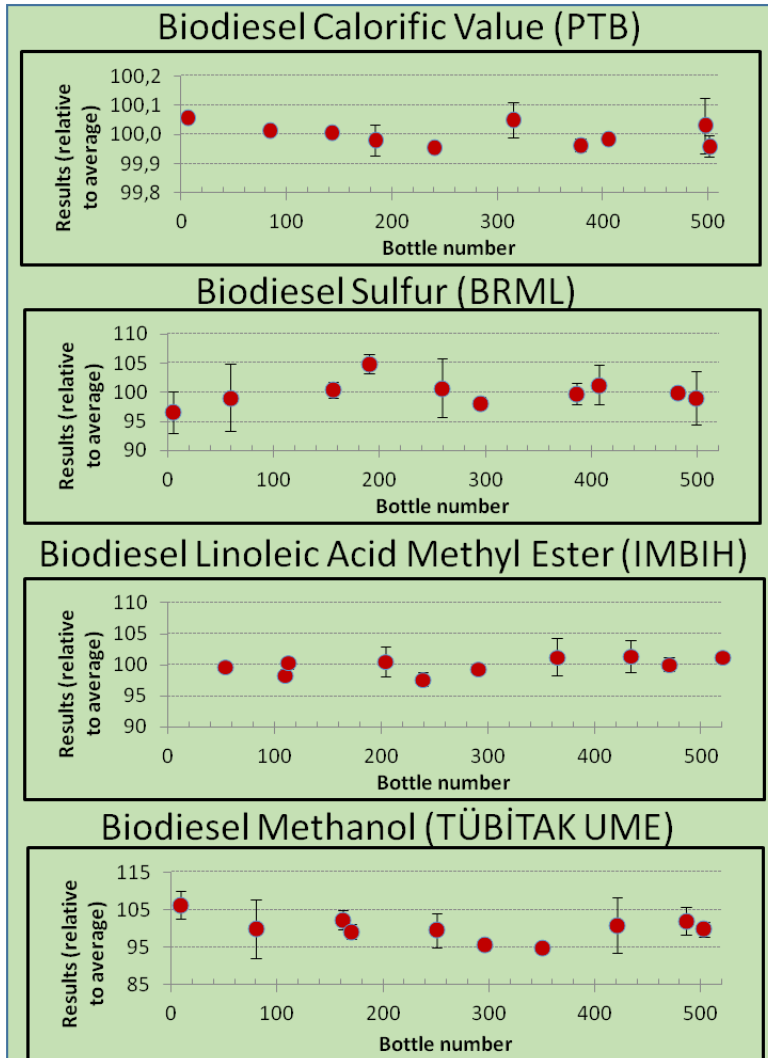
If method repeatability is not adequate to demonstrate present heterogeneity:

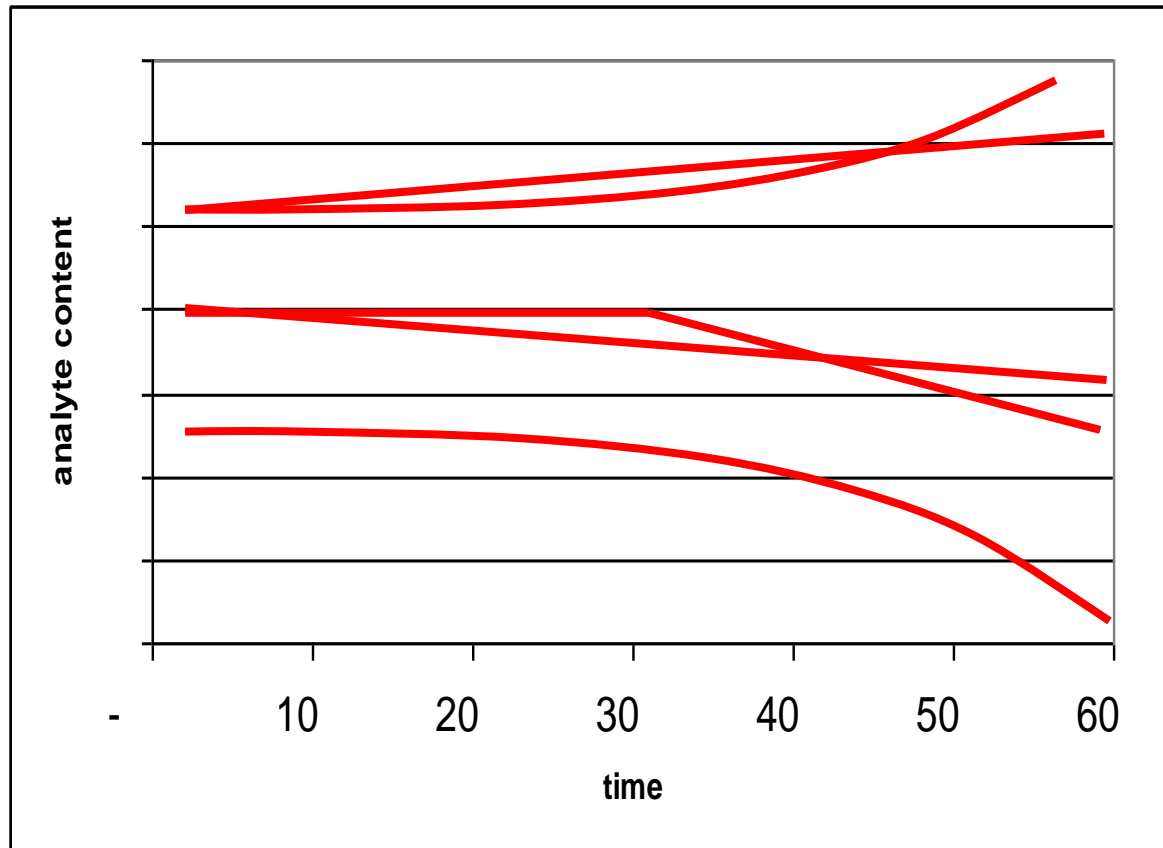
$$u_{bb,rel}^* = \frac{\sqrt{\frac{MS_{within}}{n}} \sqrt[4]{\frac{2}{\nu_{MS_{within}}}}}{\bar{y}} \quad MS_{between} < MS_{within}$$

- $MS_{within}$ : mean square within a unit from an ANOVA
- $MS_{between}$ : mean squares between-unit from an ANOVA
- $\bar{y}$ : average of all results of the homogeneity study
- $n$ : average number of replicates per unit
- $\nu_{MS_{within}}$ : degrees of freedom of  $MS_{within}$



# Homogeneity Plots





diffusion  
 analyte creation  
 controlled matrix  
 by microbes  
 degradation

consumption of an  
 antioxidant;  
 controlled analyte  
 degradation  
 autocatalytic

- **Stability Studies are necessary to find out the conditions at which degradation can be accepted as negligible.**
- **The uncertainty arised from the degradation is also estimated with these studies.**

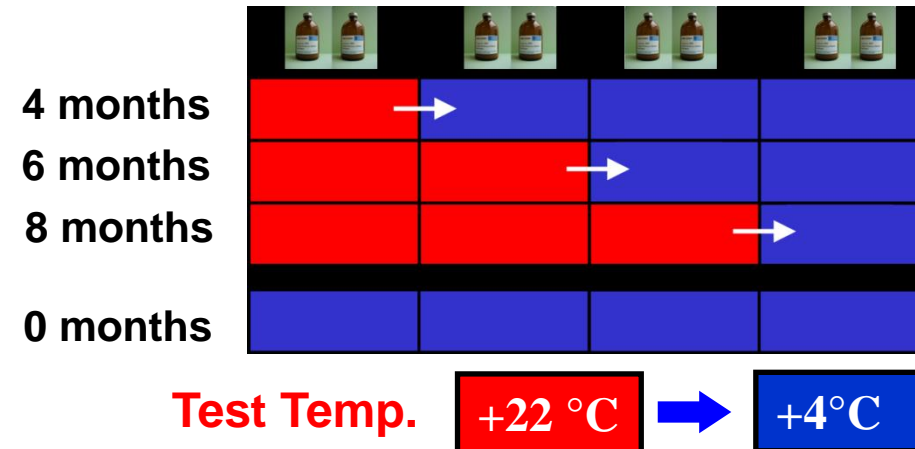
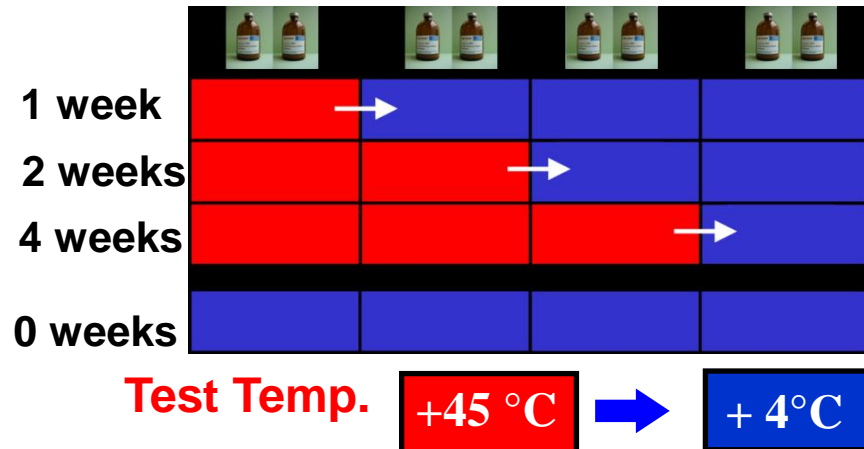
Stability during transfer

Stability during long term storage

Short Term Stability (STS)

Long Term Stability (LTS)

Isochronous Scheme



**Advantage of Isochronous Study:**

All samples are analysed the same day at the end of the study  
 ⇒ precision is not compromised by day-to-day variation



Unstability: Slope of the regression line  
Stability Uncertainty: Error of the slope

$$u_b = \frac{s}{\sqrt{\sum(x_i - \bar{x})^2}}$$

$$s = \sqrt{\frac{\sum(y_i - \hat{y})^2}{n-2}}$$

$$\hat{y} = a + bx$$

$$b = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sum(x_i - \bar{x})^2}$$

$$a = \frac{\sum y_i}{n} - b \cdot \frac{\sum x_i}{n}$$

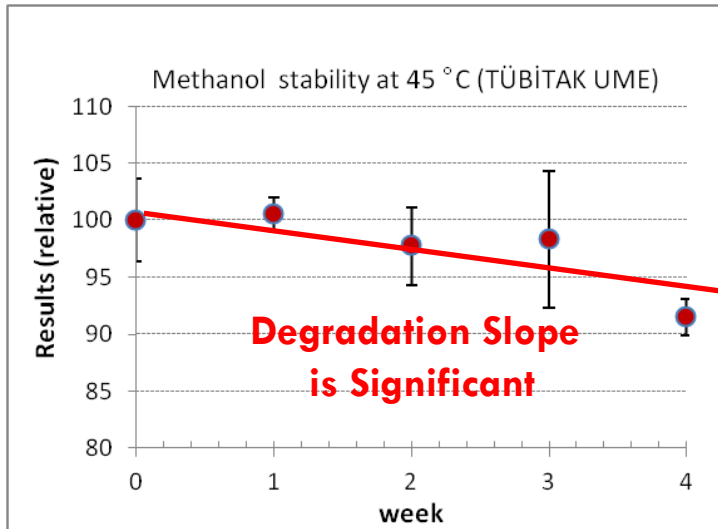
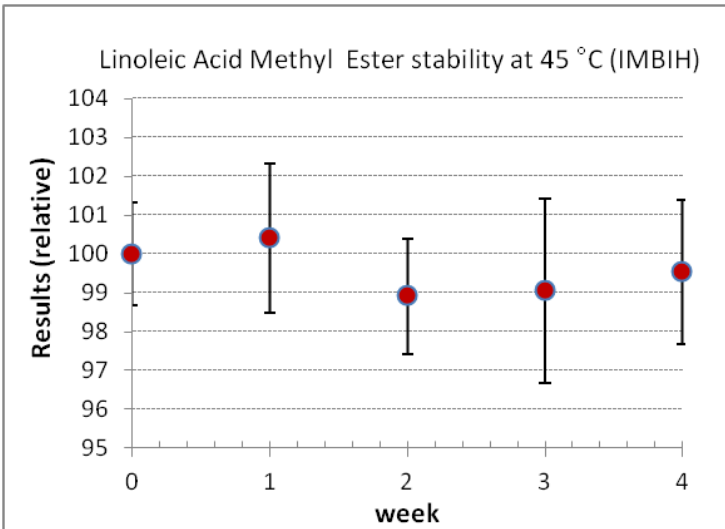
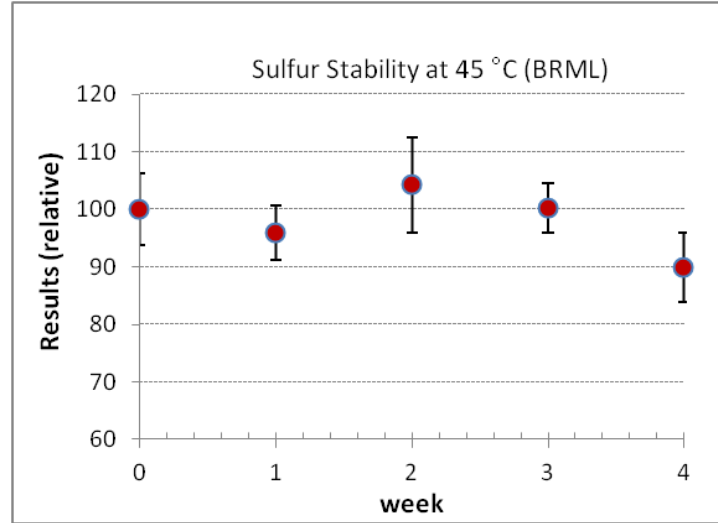
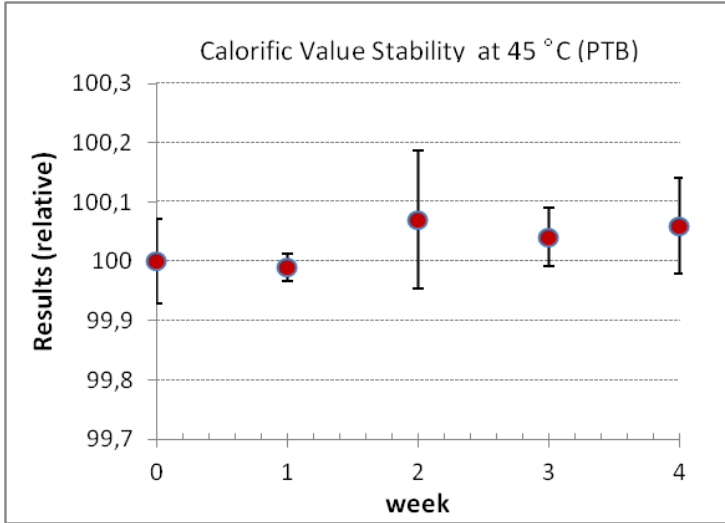
- x: Time point for measurement
- $\bar{x}$ : Mean of all time points
- n: Total number of points
- y: Measured value
- $\hat{y}$ : Mean of measured values

**$u_{STS} = \text{Transfer time} \times \text{Error of Slope}$**

**$u_{LTS} = \text{Storage time} \times \text{Error of Slope}$**

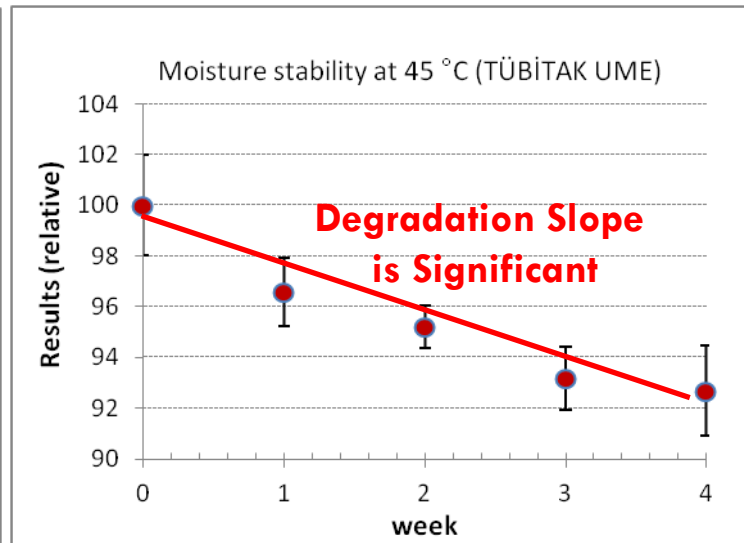
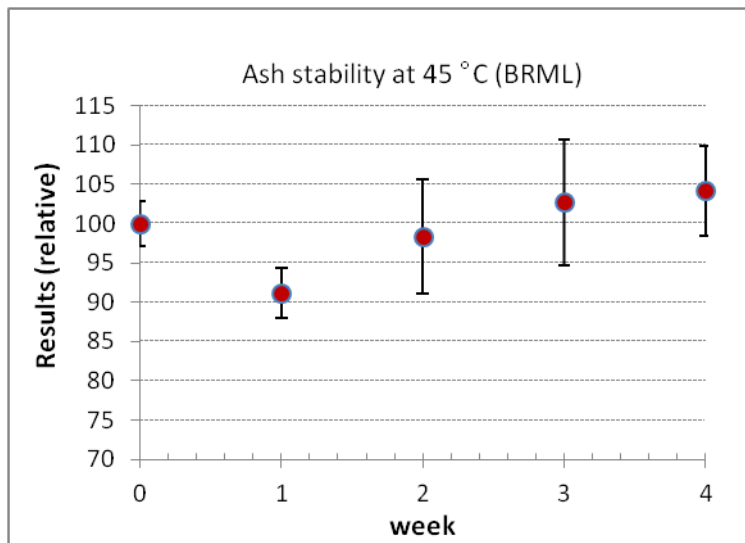
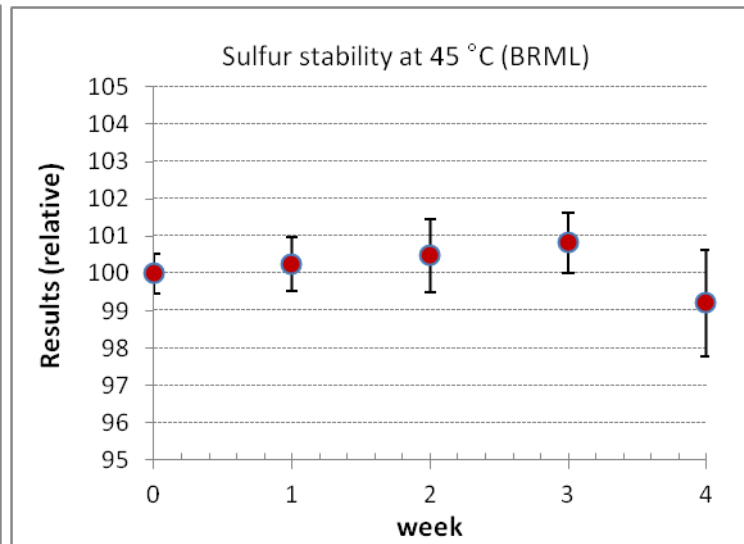
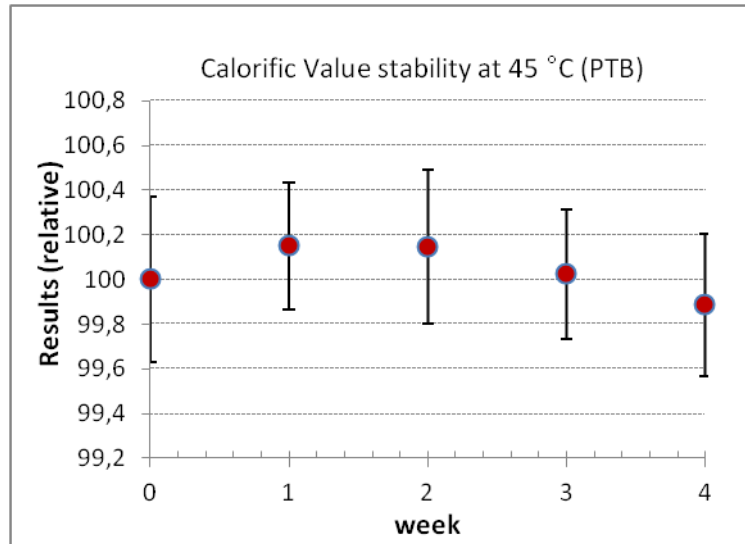
### Assumptions:

- ✓ t=0 There is no degradation
- ✓ Degradation is linear with a fixed rate
- ✓ Degradation is affecting the whole units in the same way



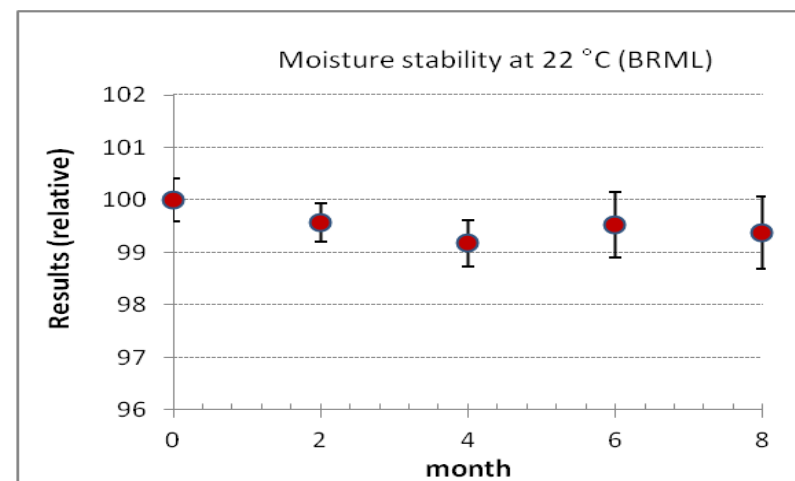
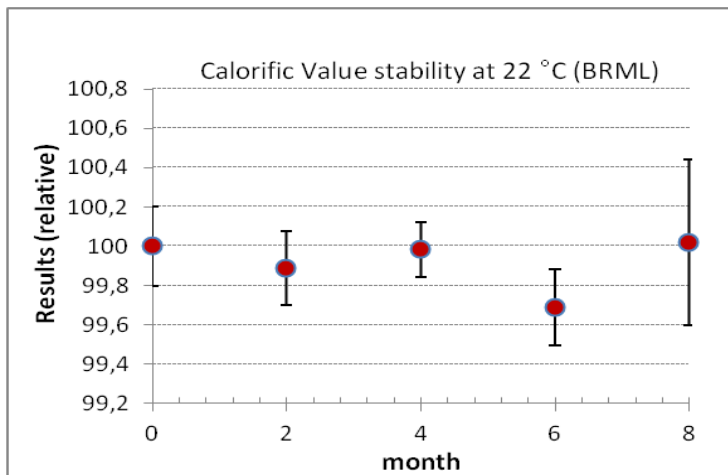
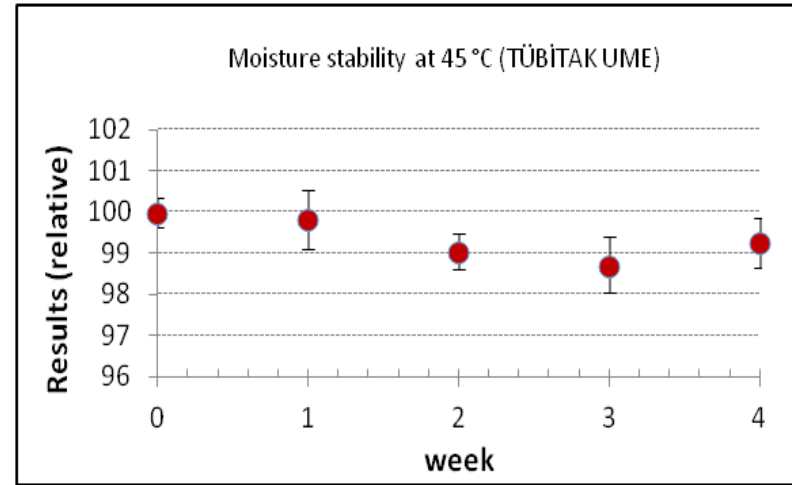
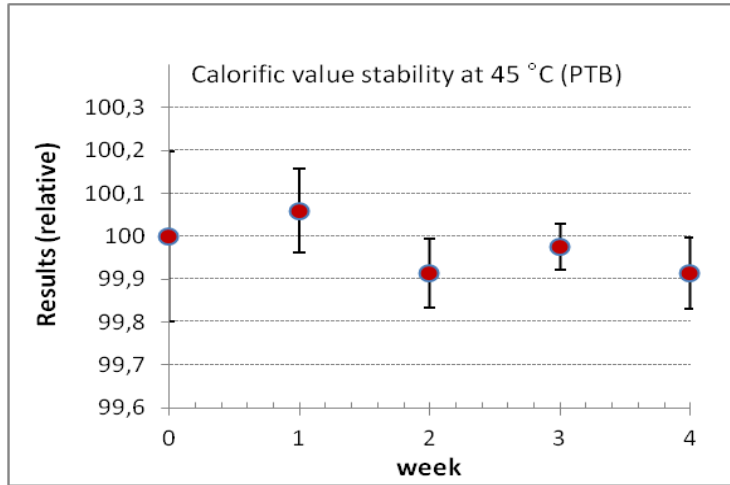
BIODIESEL		
Parameter (Lab)	Homogeneity $u_{bb,rel}$ (%)	Stability $u_{STS}$ (%) 2 weeks 45 °C
Calorific Value (PTB)	0.023	0.023
Mono-Glycerides (BRML)	0.28	0.23
Di -Glycerides (BRML)	0.45	0.36
Tri -Glycerides (BRML)	1.2	1.1
Free Glycerol (BRML)	0.84	0.63
Total Glycerol (BRML)	0.22	0.16
Linoleic acid methyl ester (IMBIH)	0.78	0.46
Methyl palmitoleate (IMBIH)	3.0	1.5
Methyl palmitate (IMBIH)	1.7	0.69
Methyl 11-octadecenoate (IMBIH)	0.82	0.41
Methyl stearate (IMBIH)	1.5	1.4
Methyl cis-11-eicosenoate (IMBIH)	3.3	1.6
Sulfur (BRML)	1.1	1.9
Calcium (BRML)	3.8	2.8
Phosphorus (BRML)	4.2	3.2
Sodium (BRML)	8.2	4.8
Potassium (BRML)	6.3	2.8
Magnesium (BRML)	3.5	3.1
Viscosity (PTB)	0.031	0.13
Density (PTB)	0.0017	0.0029
Methanol (TÜBİTAK UME)	2.2	2.5

# Wood Pellet Powder Short Term Stability Plots Homogeneity & STS Uncertainties



WOOD PELLET POWDER		
Parameter (Lab)	Homogeneity $u_{bb,rel}$ (%)	Stability 2 weeks 45 °C $u_{STS}$ (%)
Calorific Value (PTB)	0.071	0.082
Moisture (TÜBİTAK UME)	0.89	2.2
Ash (TÜBİTAK UME*, BRML**)	2.5*	1.7**
Cd (BRML)	11	6.8
Cr (IMBIH)	3.1	0.56
Cu (BRML)	4.3	2.1
Ni (IMBIH)	2.4	0.82
Pb (IMBIH*, BRML**)	5.6*	2.1**
S (BRML)	1.1	0.27
Zn (IMBIH)	2.8	2.2
Ca (IMBIH)	1.5	4.5
Fe (IMBIH)	3.6	5.5
K (IMBIH)	4.4	1.7
Mg (BRML)	10	2.6
Mn (IMBIH)	1.1	0.99
Na (IMBIH*, BRML**)	7.8*	3.8**
P (BRML)	8.8	2.0
Si (IMBIH*, BRML**)	7.0*	5.0**
Ti (BRML)	8.4	1.6

# Wood Pellet Short Term Stability Plots Homogeneity, STS, LTS Uncertainties



WOOD PELLET			
Parameter (Lab)	Homogeneity $u_{bb,rel}$ (%)	Stability 2 weeks 45 °C $u_{STS}$ (%)	Stability 1 year 22 °C $u_{LTS}$ (%)
Calorific Value (BRML*, PTB**)	0.05*	0.036**	0.22*
Moisture (TÜBİTAK UME*, BRML**)	0.25*	0.36*	0.44**



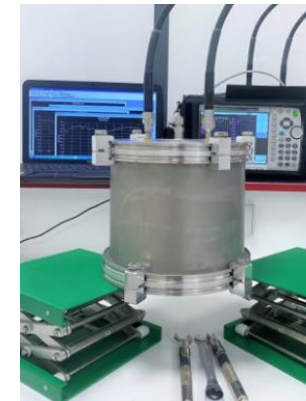
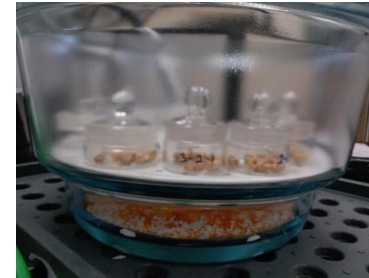
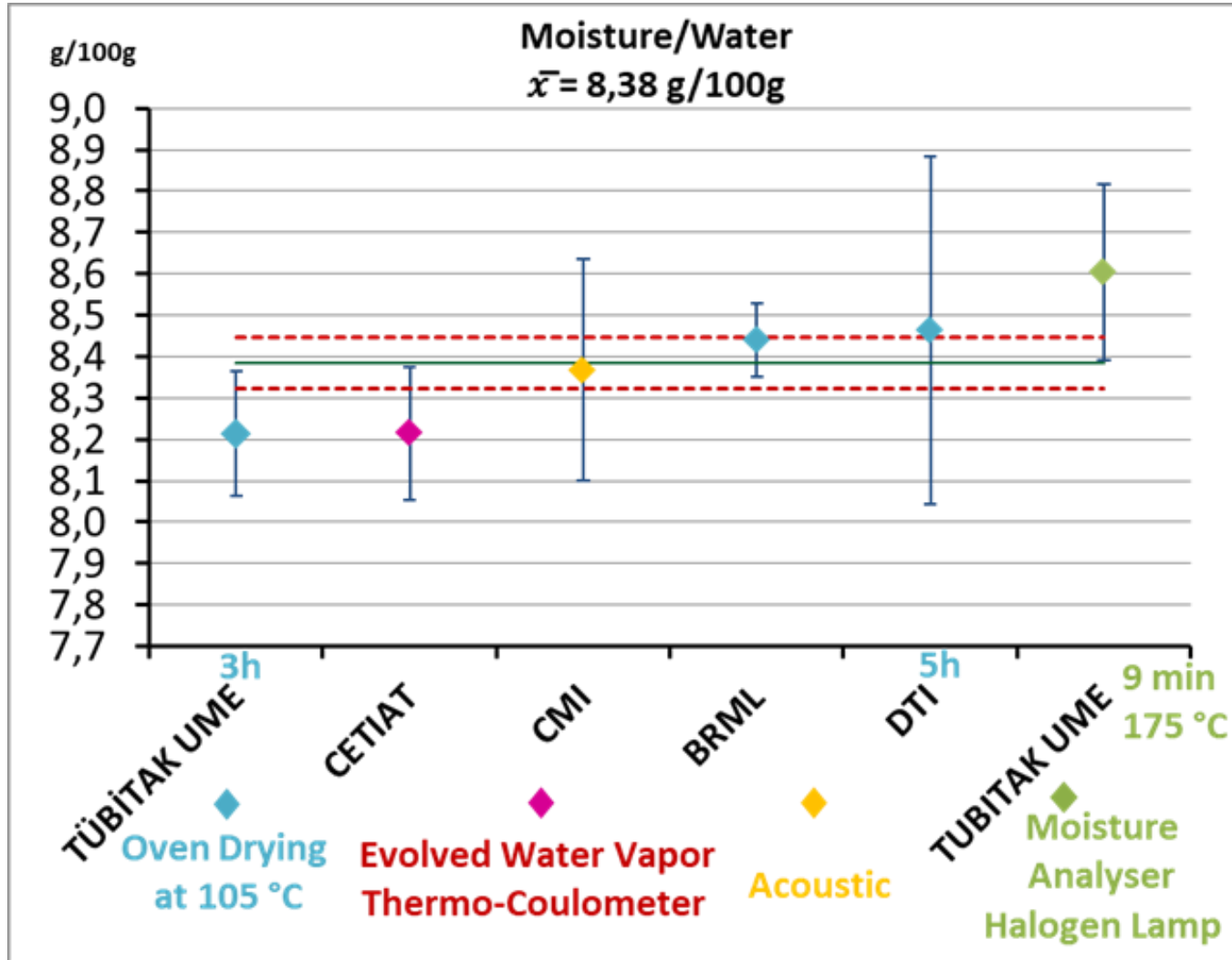
## Characterization (Value Assignment) Strategies

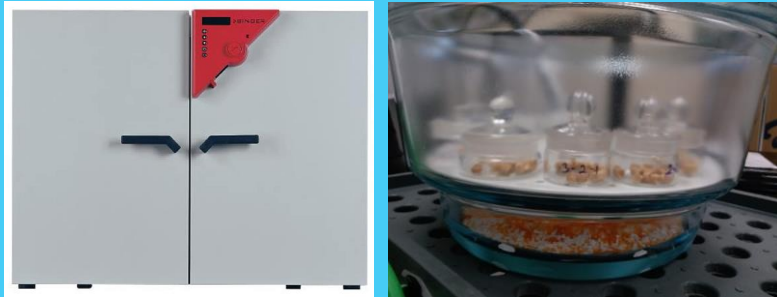
1. Using a **single reference** measurement procedure in a single laboratory  
(e.g. ID-ICP-MS method for Sulfur in Biodiesel)
2. Characterization of a **non-operationally** defined measurand using two or more methods of demonstrable accuracy in one or more **competent** laboratories  
(e.g. Cr in Wood Pellet by ICP-MS, ICP-OES and MP-AES methods)
3. Characterization of an **operationally-defined** measurand using a network of competent laboratories (e.g. Moisture in wood pellet powder by ISO-18134-3:2015 method)

## CRM Uncertainty

$$U_{CRM} = k \cdot \sqrt{u_{char}^2 + u_{hom}^2 + u_{sts}^2 + u_{lts}^2}$$

# Wood Pellet Moisture/Water Characterisation Study





ISO-18134-3:2015 Method, Temperature: **105 °C**  
 Sample Heating Time: **3-5 hours**  
 Time needed for 36 Measurements : **7- 8 hours**  
 Total Number of Weighings: **108-180**  
 Total Number of weighing bottles: **36**  
 Requires: **dessicator with dessicant**

Wood Pellet Pow:  $u_{\text{hom}}$  (%): **0.89**,  $u_{\text{STS}}$  (%): **2.2**

Wood Pellet  $u_{\text{hom}}$  (%): **0.25**,  $u_{\text{STS}}$  (%): **0.36**



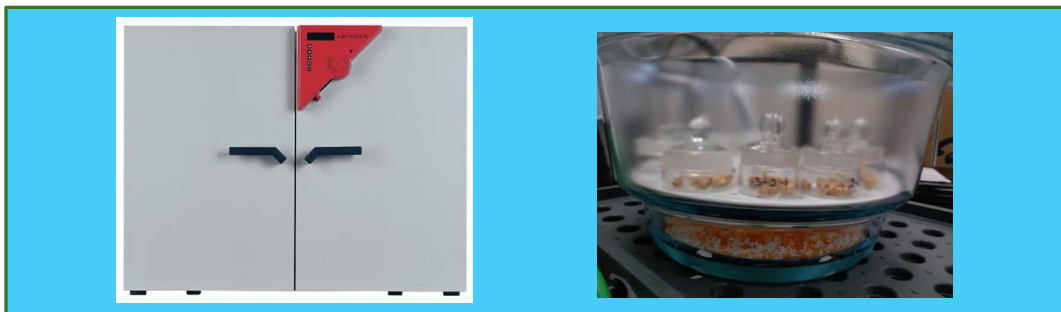
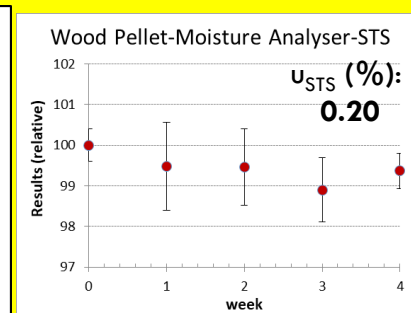
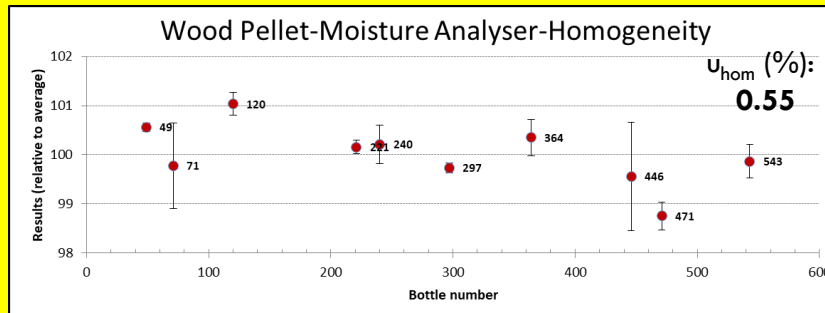
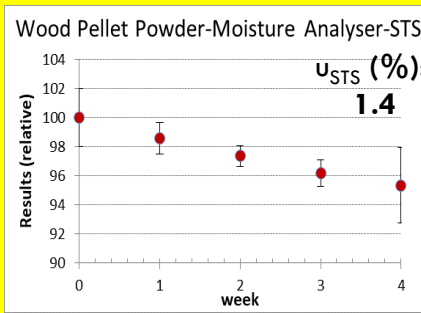
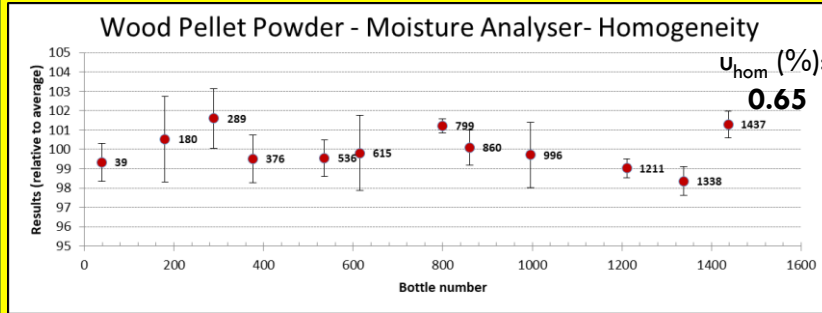
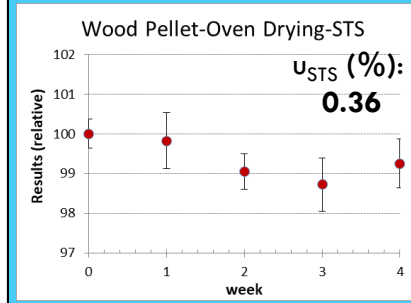
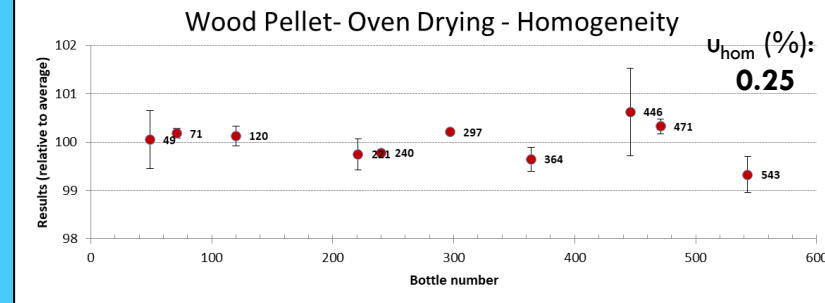
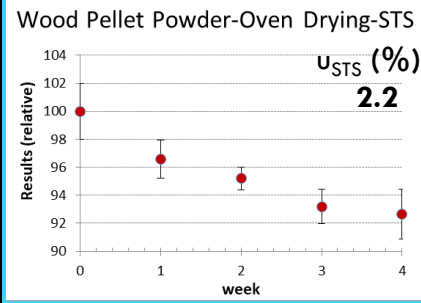
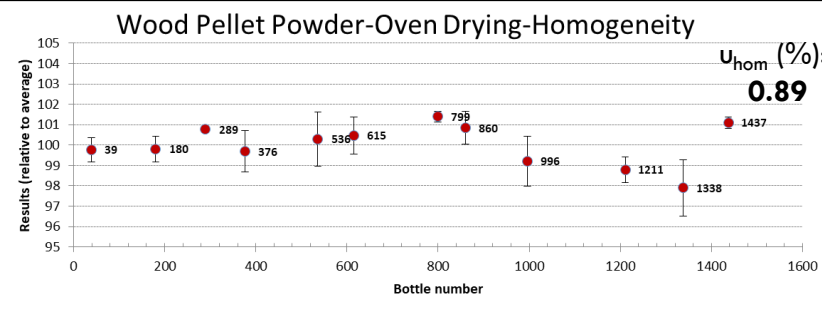
Temperature: **105 °C**: pellet powder, **175 °C** : pellet  
 Sample Heating Time: **3 min**-powder, **8 min** for pellet  
 Total Time needed for 36 Measurements : **3h/6h**  
 Total Number of Weighings: **36**  
 Total Number of aluminum weighing plates: **2**  
**No** dessicator with dessicant is required

Wood Pellet Powder:  $u_{\text{hom}}$  (%): **0.65**,  $u_{\text{STS}}$  (%): **1.4**

Wood Pellet :  $u_{\text{hom}}$  (%): **0.55**,  $u_{\text{STS}}$  (%): **0.20**

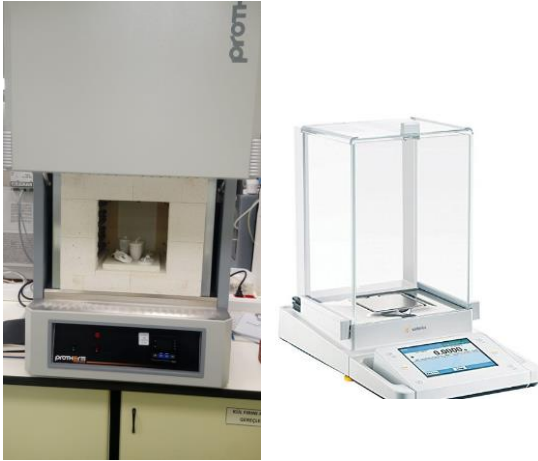
## UME BIOFMET CRM 02-WOOD PELLET POWDER

## UME BIOFMET CRM 03-WOOD PELLET





**ISO 18122:2022**  
 Defines use of a balance capable of reading to the nearest **0.1 mg**



## Balance with 0.1 mg Readability

	Bottle No	Weight Empty bottle (g)	Weight Empty bottle + Sample (g)	Weight after Ashing (g)	Ash Content[Wet] (g/100g)
1	39-1	28,8251	29,7935	28,8265	0,145
2	180-1	30,1776	31,1082	30,1792	0,172
3	289-1	30,2975	31,2354	30,2990	0,160
4	376-1	31,4041	32,354	31,4063	0,232
5	536-1	30,3882	31,3377	30,3901	0,200
6	615-1	30,5544	31,4719	30,5557	0,142
7	799-1	30,9556	31,9901	30,9575	0,184
8	860-1	30,8452	31,7919	30,8474	0,232
9	996-1	28,9917	29,9868	28,9941	0,241
10	1211-1	28,3408	29,285	28,3433	0,265
11	1338-1	31,0568	31,9895	31,0590	0,236
12	1437-1	30,8627	31,8441	30,8649	0,224
13	39-2	28,991	30,038	28,9930	0,191
14	180-2	30,862	31,8884	30,8634	0,136
15	289-2	30,5537	31,5922	30,5541	0,039
16	376-2	30,3876	31,3916	30,3888	0,120
17	536-2	28,3405	29,4019	28,3422	0,160
18	615-2	31,0576	32,0896	31,0581	0,048
19	799-2	30,1771	31,2403	30,1796	0,235
20	860-2	31,405	32,4303	31,4058	0,078
21	996-2	28,8245	29,831	28,8264	0,189
22	1211-2	30,8444	31,9999	30,8473	0,251
23	1338-2	30,9544	32,0149	30,9571	0,255
24	1437-2	30,2976	31,3364	30,3001	0,241
			<b>Avg</b>	<b>0,182</b>	<b>g/100g</b>
			<b>SD</b>	<b>0,065</b>	<b>g/100g</b>
			<b>RSD</b>	<b>35,5</b>	<b>%</b>



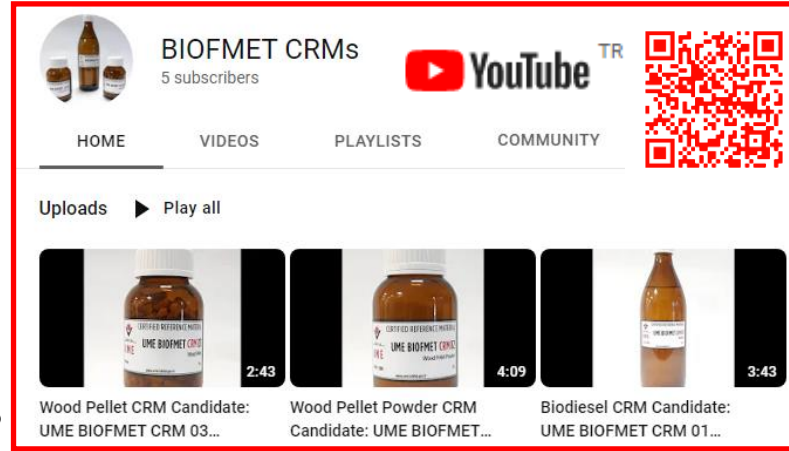
## Balance with 0.01 mg Readability

	Bottle No	Weight Empty bottle (g)	Weight Empty bottle + Sample (g)	Weight after Ashing (g)	Ash Content[Wet] (g/100g)
1	39-1	31,05674	32,51881	31,06019	0,236
2	180-1	31,40455	32,85538	31,40780	0,224
3	289-1	30,86251	32,31448	30,86572	0,221
4	376-1	28,34106	29,79704	28,34455	0,240
5	536-1	30,55297	32,00488	30,55652	0,245
6	615-1	30,29754	31,75746	30,30095	0,234
7	799-1	30,95462	32,41181	30,95830	0,253
8	860-1	30,84532	32,30066	30,84850	0,219
9	996-1	28,99206	30,44316	28,99520	0,216
10	1211-1	28,8249	30,2739	28,82820	0,228
11	1338-1	30,38783	31,84003	30,39122	0,233
12	1437-1	30,1776	31,62817	30,18132	0,256
13	39-2	31,40414	32,8573	31,40707	0,202
14	180-2	30,8443	32,2957	30,84777	0,239
15	289-2	28,99155	30,44392	28,99451	0,204
16	376-2	31,05622	32,50509	31,05921	0,206
17	536-2	30,86194	32,32111	30,86538	0,236
18	615-2	30,17735	31,6304	30,18041	0,211
19	799-2	28,34089	29,7997	28,34444	0,243
20	860-2	30,55285	32,00395	30,55650	0,252
21	996-2	30,29676	31,75162	30,30060	0,264
22	1211-2	30,95454	32,40752	30,95764	0,213
23	1338-2	28,82443	30,28181	28,82813	0,254
24	1437-2	30,38738	31,84337	30,39121	0,263
			<b>Avg</b>	<b>0,233</b>	<b>g/100g</b>
			<b>SD</b>	<b>0,019</b>	<b>g/100g</b>
			<b>RSD</b>	<b>8,0</b>	<b>%</b>





TÜBİTAK UME BIOFMET project RM processing team



## Acknowledgment

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- Project Partners & Collaborators

### • TÜBİTAK UME Staff:

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Hasibe Yılmaz (Methanol Measurements)

Adnan Şimşek (C,H,N Measurements)

Gökhan Aktaş, Hatice Altuntaş (Processing & Stability Tests)

Tülin Erdoğan (Ash & Ion Measurements)

Uğur Akkaya (Project Logo Design)

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# Thank you for your attention!

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