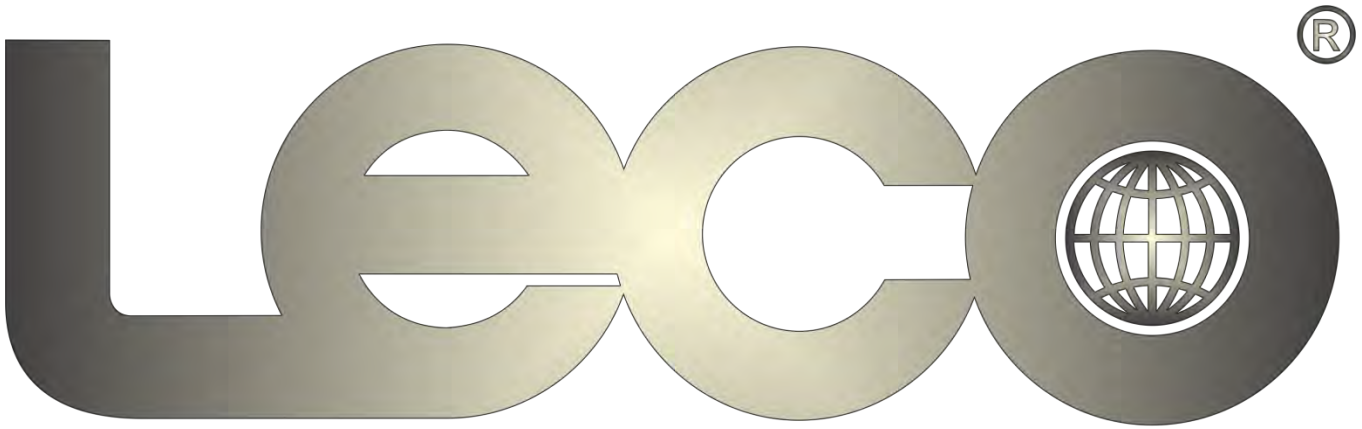


Delivering the Right Results

VERENIGING VOOR NUTRIENTEN ANALYSE

VNA Symposium at DSM Deinze 05.12.2013

Leco®
Delivering the Right Results

Presentation

- LECO Background
- Nitrogen/Protein Determination Techniques
- LECO Nitrogen Determination
  - Vertical Furnace (FP-628™)
  - Horizontal Furnace (TruMac™ N)

by Dipl.- Ing. Beate Boisten

LECO Background

- Privately owned U.S. Company for 77 years
- Headquartered in Saint Joseph, MI

- Diverse Instrumentation offerings
  - Organic and Inorganic Elemental Analysis
  - Metallography
  - Microscopy and Image analysis
  - Mass Spectrometry (TOF)

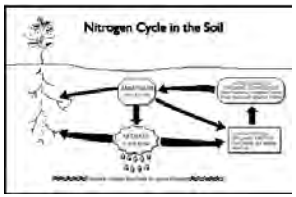
General Markets 2011-.wmv

- 25 LECO subsidiaries representing over 100 countries

Why Nitrogen / Protein Determination?

- Quantitative Analysis
  - Nutritional labeling (protein) for foods and feeds
  - Material characterization - education/research, commercial and regulatory
  - Soil/plant science

Dietary Fiber 0g	0%
Sugars 5g	
Protein 5g	
Vitamin A	4%
Vitamin C	2%
Calcium	20%
Iron	4%



- Process and Quality Control
  - Grade and value for feeds
  - Food, agricultural and chemical processing
    - Milling applications (starch, flour, oilseeds)
    - Chemical and Specialty materials processing (plastics, resins, adhesives, etc.)

Primary Techniques for Determining Nitrogen

- **Kjeldahl** and **Dumas** based techniques
  - Most common nitrogen determination techniques
  - Determine nitrogen content in a sample to calculate protein content
    - ***Nitrogen*** concentration ***is multiplied by a factor*** dependent on a specific matrix (typically between 5.70 and 6.38) ***to calculate the*** concentration of ***raw protein*** in the sample.
  - **Dumas** technique is commonly referred to as **"Combustion"**

Techniques for Determining Nitrogen

- **Kjeldahl** Nitrogen Determination
  - Classical wet chemical **digestion** and **titration-based** method long used (developed in 1883) and widely accepted; considered the primary Nitrogen determination method
  - Several hundred mg- to gram-sized sample is digested in **concentrated sulfuric acid with a catalyst**; **nitrogen is converted to ammonium sulfate**
  - Concentrated sodium hydroxide **(NaOH) is added** to form **ammonia**, which is distilled into standard acid for **quantification by titration**

Techniques for Determining Nitrogen

- **Kjeldahl** Nitrogen Determination

  - **Disadvantages**
    - **Most effective** Kjeldahl **catalysts** (containing **mercury or selenium**) are not used due to **environmental and health** concerns
    - **$CuSO_4$/TiO_4** based Kjeldahl catalyst is typically utilized, resulting in a **lower Nitrogen recovery** during digestion step and a low Nitrogen bias
    - **Long analysis times** (several hours)
    - **High cost-per-analysis**
    - Occupational health and safety issues
      - **Strong acid** and base reagents – boiling reagents required
      - **Fume handling**
      - **Waste disposal**
    - Requires analytically trained technician

Nitrogen determination with Dumas

Dumas principle

→ Sample combustion by pure oxygen
→ Combustion / oxidation of the protein-N to NO_x
→ Complete reduction of NO_x to N_2 by copper metal
→ Removal of "disturbing" gases (excessive O_2; CO_2, Water, …)
→ Detection of the nitrogen with TCD (thermal conductivity detector)
→ Calculation protein % = factor x nitrogen%

– Since ~ 25 years established in the market
– Meanwhile standards like : DIN, ISO, AOAC, §64LFBG etc…..
  => Referenced method

Norms

AOAC

Instruments	Method	Constituent	Materials
FP628, FP-528 TruSpec® N, TruMac® N	990.03	Crude Protein	Animal Feed
	992.15		Meat/Meat Products & Petfoods
	992.23		Cereal Grain & Oil Seed Products
	993.13	Nitrogen	Fertilizer
	997.09	Nitrogen/ Protein	Beer, Wort, Brewing Grains (Total Protein by Calculation)

ISO

Instruments	Method	Elements	Materials
FP628, FP528, TruSpec N, TruMac N	14891	Total Nitrogen/Protein	Milk and Milk Products
FP628, FP528, TruSpec N, TruMac N	16634	Total Nitrogen/Protein	Food Products, Oilseed, and Animal Feeds

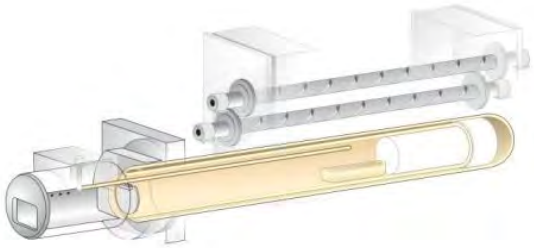
- § 64 LFBG : German official food methodology
- VD LUFA standards : Agricultural Society of Germany (different standards)

LECO Combustion Nitrogen Determination

- **FP-628™** utilizes a **vertical furnace**
- Sample mass **50 mg up to 1 g**
- Quartz furnace tube with a maximum temperature of 1050°C
- Tin capsules or foils used to encapsulate sample
- Reticulated crucible collects ash from sample and tin cap or foil
  - **Crucible lifetime** matrix dependent (**200-400 samples**)

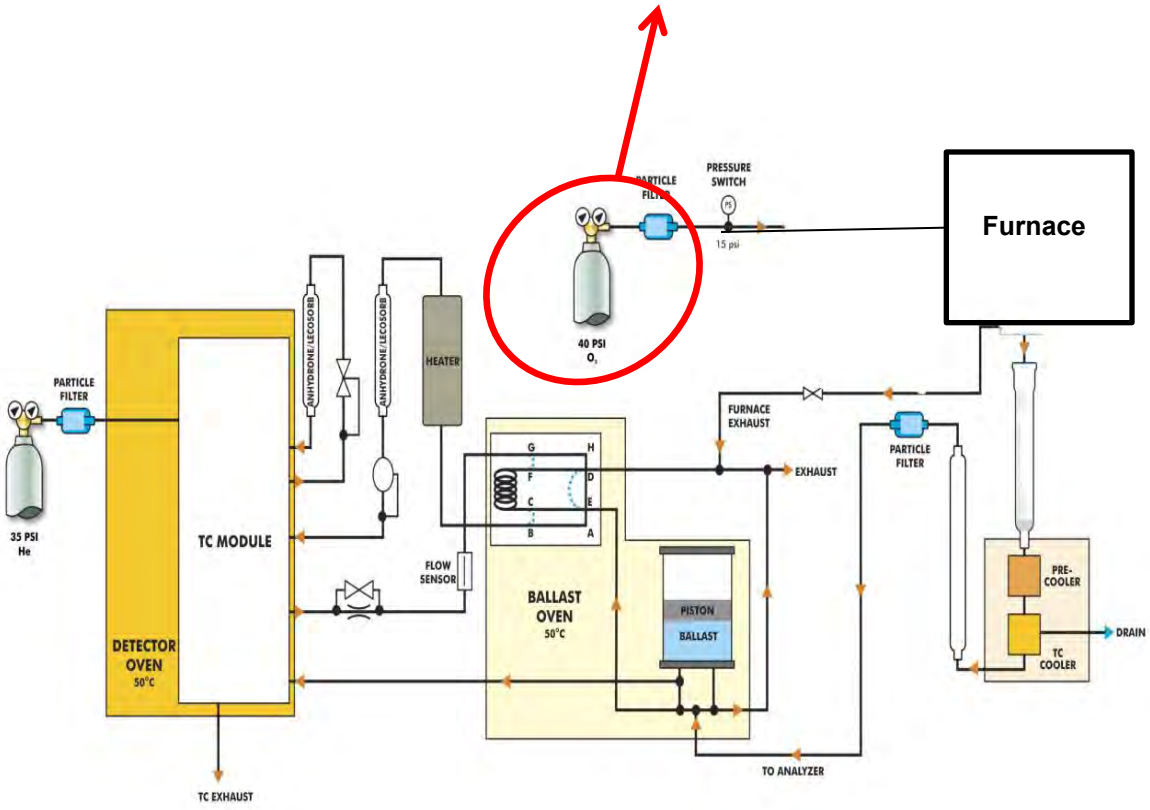
LECO Combustion Nitrogen Determination

- **TruMac N** utilizes a **horizontal furnace**
- Sample mass **50 mg up to 3 g**
- Ceramic furnace
  - Designed for handling macro sample combustion
  - Maximum Temperature of 1450°C
- Large reusable ceramic boats
  - Facilitate macro sample handling
  - Retain **sample ash** for post analysis **removal**



LECO Combustion Nitrogen Determination

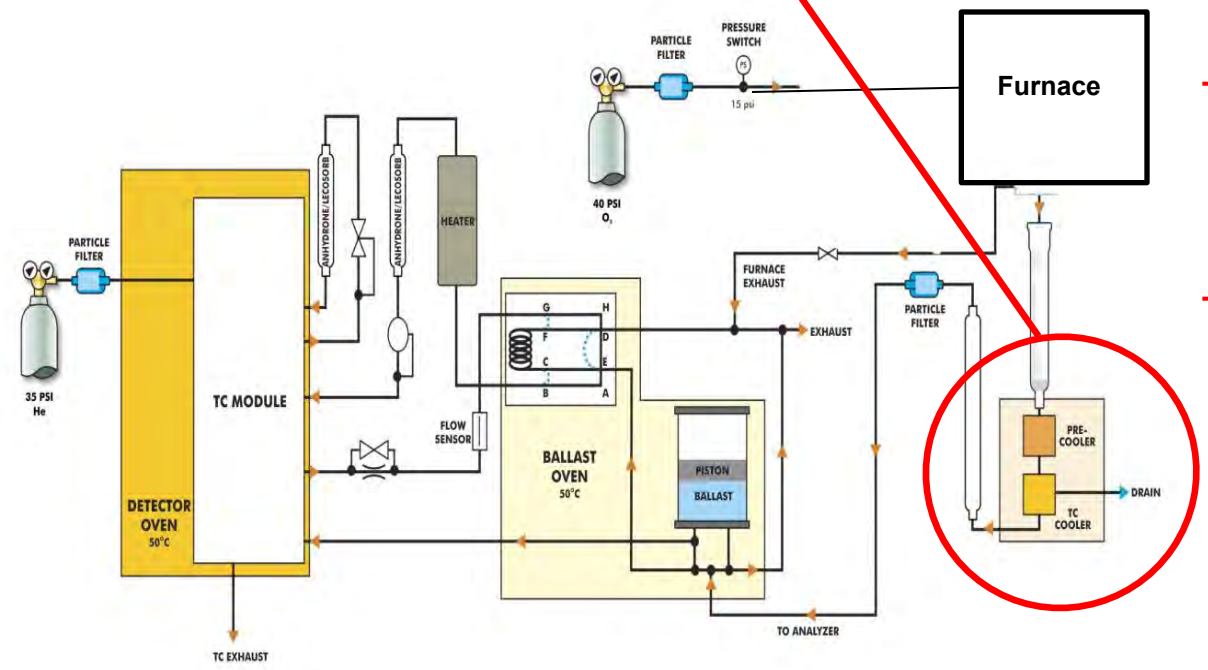
- Both **vertical** and **horizontal furnace** designs utilize a *Pure Oxygen Environment in Furnace*



- Ensures complete combustion
- Eliminates the need for additional chemical or metal oxidizers and reagents in flow path
- Sample matrix independent (feeds, food, bones, meat, etc.)
- Increases speed of combustion
- Allows large sample mass capability

LECO Combustion Nitrogen Determination

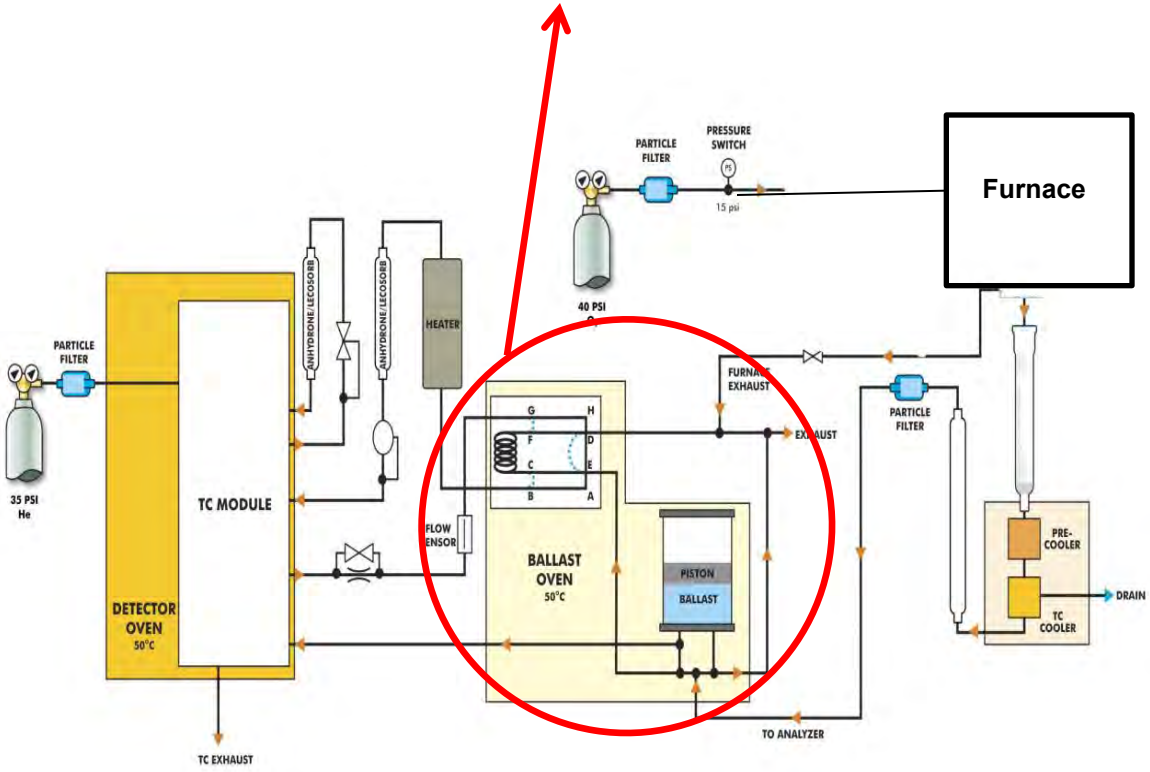
- Both **vertical** and **horizontal** furnace designs utilize a *Thermo Electric Cooler*



- Removes moisture from combustion gas by cooling/condensing moisture
- Eliminates the need for chemical reagents (Anhydrone) to remove moisture from combustion gas
- Removes other unwanted combustion gases via interaction with condensing moisture
  - Acid gases (sulfur, chlorine, halogens, etc.)
  - Combustion particulates

LECO Combustion Nitrogen Determination

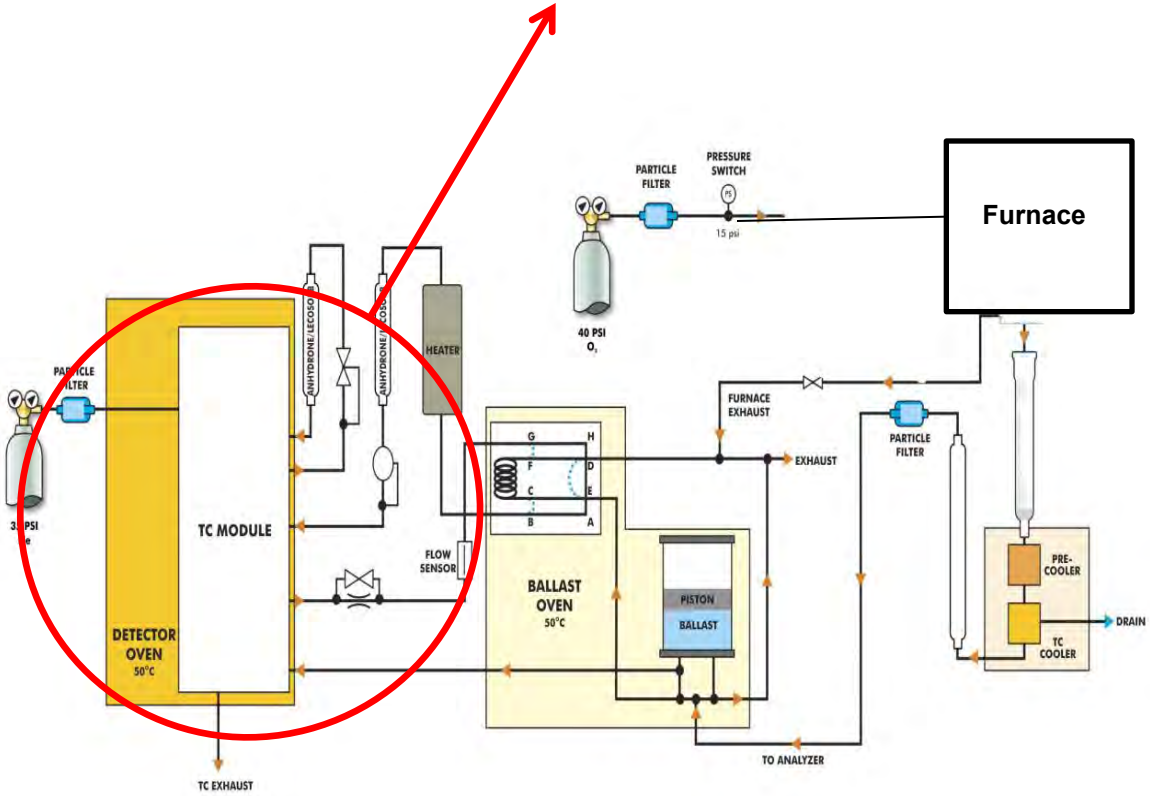
- Both **vertical** and **horizontal** furnace designs utilize a *Combustion Gas Collection* and *Aliquot Dose System*



- Collects and equilibrates all combustion gas for aliquot collection
- Eliminates treating the whole combustion gas with reduction reagents and carbon scrubbers
- Greatly extends lifetime of carbon scrubber and reduction reagents
  - Allows large sample mass capability
- Greatly lowers the cost-per-analysis

LECO Combustion Nitrogen Determination

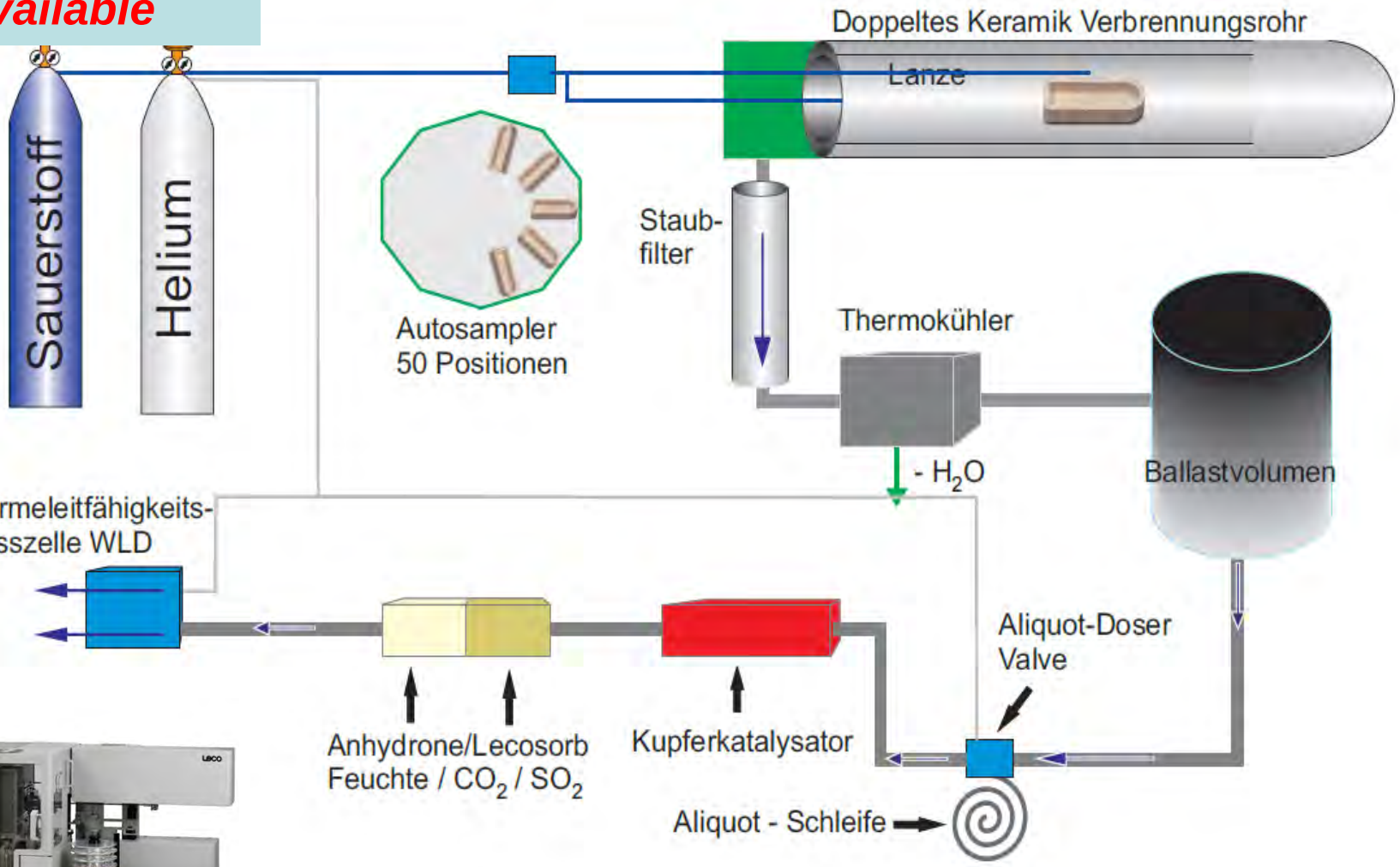
- Both **vertical** and **horizontal** furnace designs utilize a *Thermal Conductivity Cell for Nitrogen Detection*



- Wide linear dynamic range
- Low noise and drift
- Low helium consumption

Argon as carrier gas available

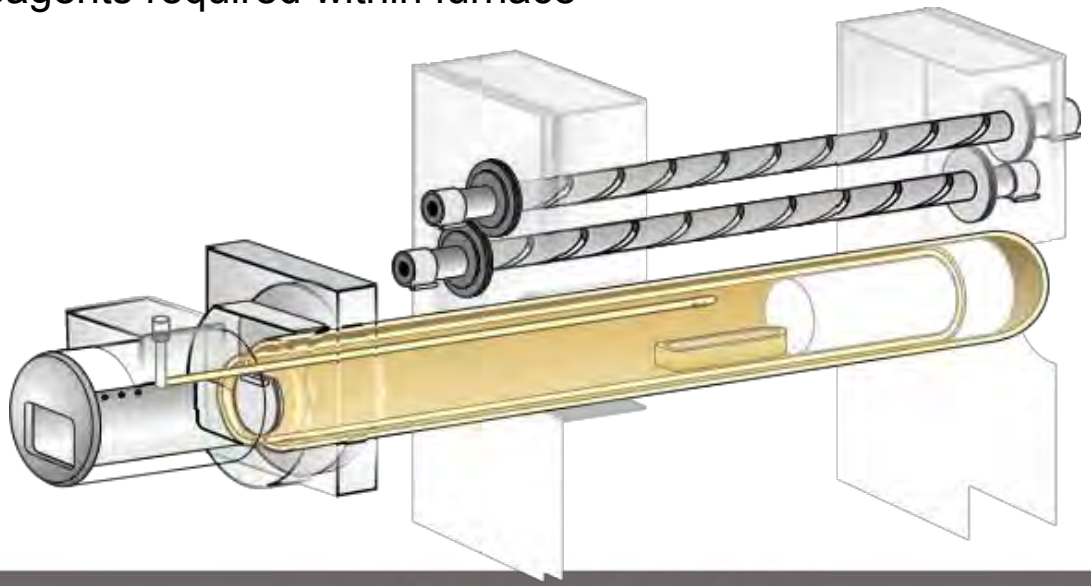
Gas flow diagram TruMac N



Delivering the Right Results

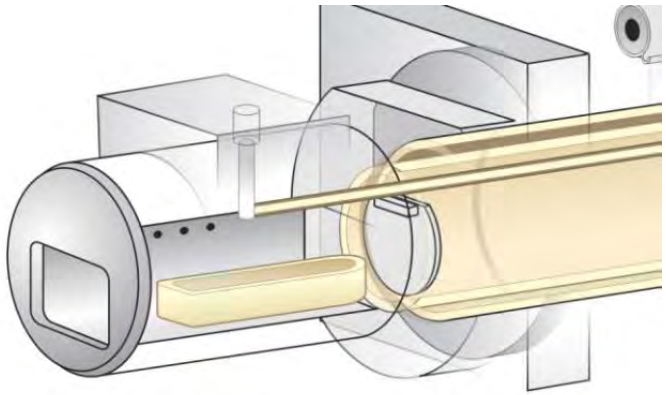
TruMac N

- Ceramic horizontal furnace designed for macro samples
  - Robust design utilizes 6 heating elements for increased reliability and heating efficiency with a maximum temperature of 1450°C
  - **Pure oxygen** environment ensures **complete combustion** of macro samples
  - Ceramic **lance** directs additional oxygen flow **directly onto the sample**
  - No reagents required within furnace



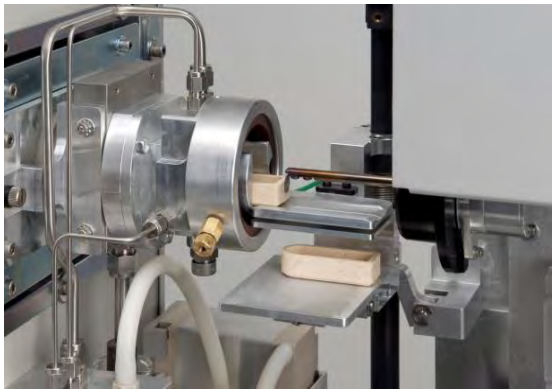
TruMac N

- **Open ceramic** boats provide sample **surface area** enhancing:
  - Efficient atmospheric purging of macro samples
  - Complete combustion of heterogenous macro samples



TruMac N

- Automated sample **ash removal** from furnace
- Sample ash is retained and removed in ceramic boat post analysis
  - Furnace remains free of sample ash debris and residue build up
  - Reduces the need for routine furnace maintenance
  - Eliminates the need for a furnace crucible



TruMac N Advantages

- The TruMac N **horizontal furnace** design offers additional benefits

  - Ability to handle macro samples **up to 3 g**, ideal for:
    - **Heterogeneous** and **difficult to prepare samples** (meats and feeds, etc.)
    - **Low-level nitrogen** samples (starches, slurries, wastewater, etc.)
    - **Low density or bulky samples** (filter papers/bags, celite)
  - Increased **ease of sample handling** and preparation
    - Large **reusable ceramic boats**
  - Automated sample **ash removal** from furnace

TruMac N Specifications

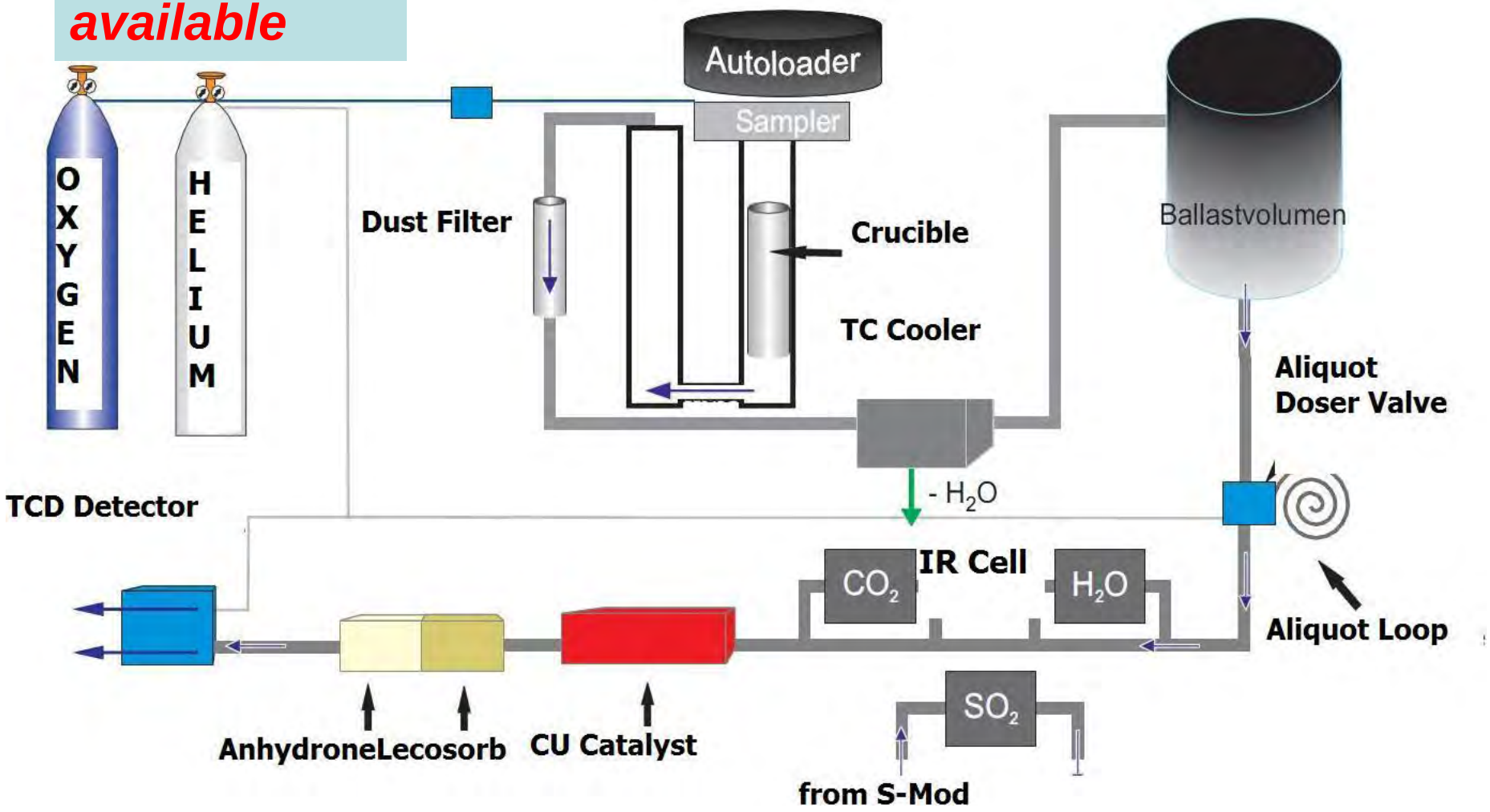
- Instrument Range @ 1.0 g*
  - Nitrogen — 20 ppm or 0.002% to 30% (0.02 mg to 300 mg Nitrogen)

**Adjusting sample size may extend instrument range*

- Precision Range @ 1.0 g
  - Nitrogen — 10 ppm or 0.3% RSD (whichever is greater)
- Analysis Time — 4 minutes nominal
- Sample Size — Up to 3.0 g, 1.0 g nominal
- Furnace Resistance — 1450°C max (1050°C nominal)
- Operational Control — Windows®-based software on an external PC
- Autoloader — 50-position
- Electrical Power Requirements
  - Instrument — 230 V~, 50/60 Hz, single phase
  - Autoloader — 1150/230 V~, 50/60 Hz, single phase

Argon as carrier gas available

Gas flow diagram FP-628 / CHNS-628



FP-628

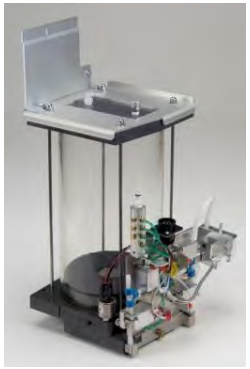
- A sample is weighed into a foil or capsule and loaded into autoloader

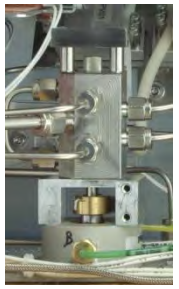
- Sample is loaded into purge chamber removing atmospheric gas
- Sample introduced to furnace

FP-628

- Combustion gases are swept from the furnace into the thermo electric cooler
- Moisture efficiently removed without the use of chemical anhydrone

- Combustion gas is swept onto the combustion gas collection and aliquot collection system

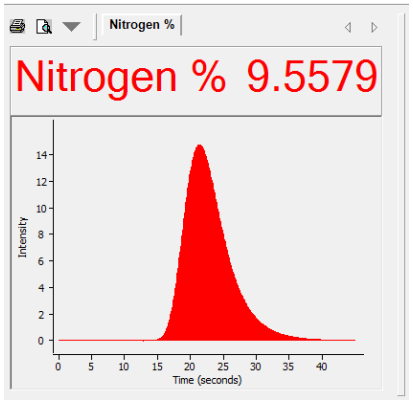




FP-628

Aliquot gas is swept through a reduction reagent tube (lifetime ~ 600 analyses) then passes through Lecosorb and Anhydrone reagent tube

The aliquot gas passes through a thermal conductivity cell for nitrogen detection



FP-628 Specifications

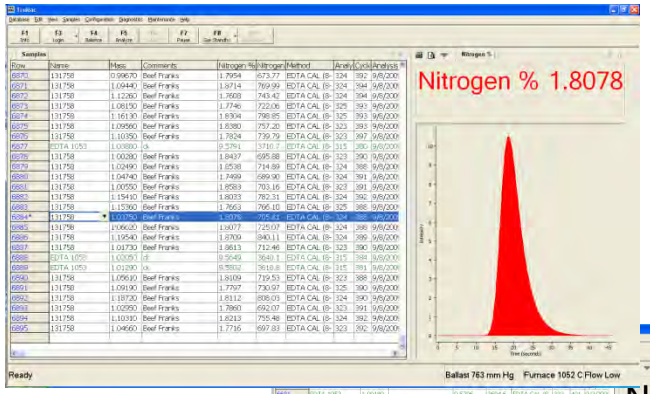
- Instrument Range
  - Nitrogen 0.04 mg to 50 mg Nitrogen; 80 ppm – 10 % Nitrogen @ 500 mg

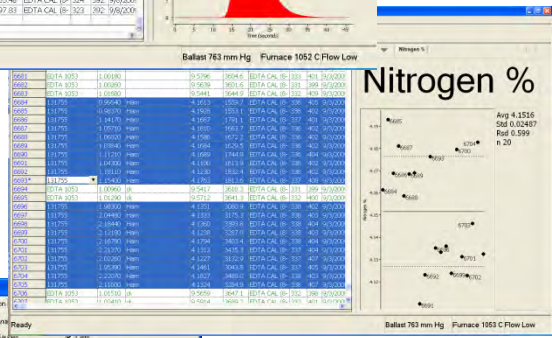
  Precision Range
  - Nitrogen 0.02 mg Nitrogen or 0.5% RSD (whichever is greater)
- Analysis Time 4.5 minutes nominal
- Sample Mass
  - N up to 750 mg, 500 mg nominal
- Autoloader 30-position, expandable up to 120

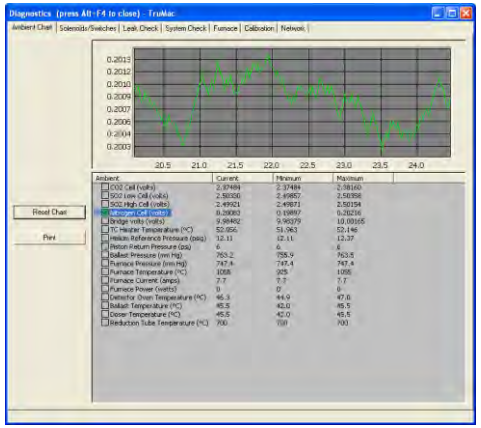
- FP628 range:
  - 500 mg sample mass
    - Nitrogen (80ppm to 10%)
      - Low end = (0.04 mg N / 500 mg sample) * 100 = 0.08% or **80 ppm** N
      - High end = (50 mg N / 500 mg sample) *100 = **10% N**

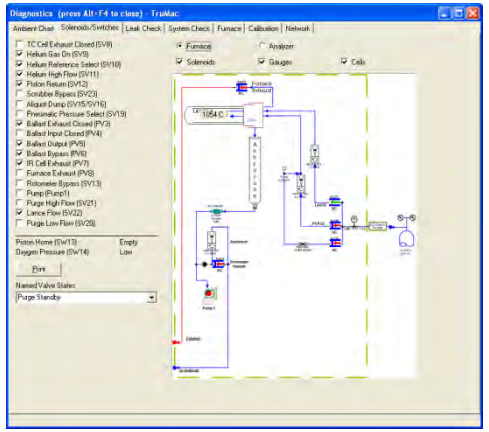
LECO Software

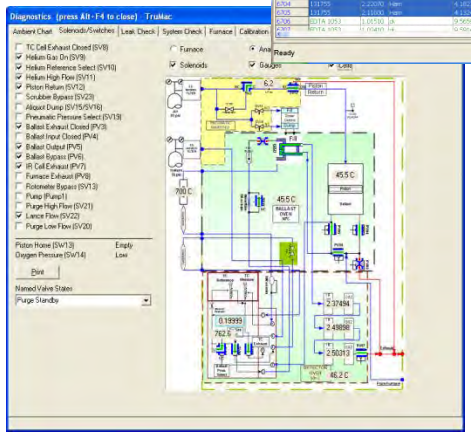
- LECO Windows-based operating software
  - Monitor real-time internal component readings
  - Expanded interactive diagnostic screens aiding
    - Troubleshooting
    - Improve serviceability











LECO Combustion Nitrogen Determination

- **extended reagent lifetime, less instrument downtimes, low cost-per-analysis!**

  - **Exclusive** use of **Oxygen** within Combustion Furnace results in:
    - Ensures **complete combustion** and superior recovery of elements
    - **Speeds combustion**
    - Allows for use of **macro sample mass**
    - **No need for other expensive reagent oxidizers** within combustion system

  - **Ballast and Aliquot** Combustion Gas Handling system
    - **Allows** for the exclusive **use of pure oxygen** in the furnace (no soot!)
    - **Eliminates treating the whole combustion gas** with reduction reagents and carbon scrubbers (10 ml instead of 5-10 l)
    - Significantly **extends lifetime of reduction reagents Cu (~600 analyses with one small tube and carbon scrubber**

TruMac N Data

- Flour Matrix Sample Data
  - ~0.5 g sample mass

TRU MAC

Wheat Flour Data

Name	Description	Mass	Nitrogen %	Protein %
502-274 1010	Wheat Flour	0.51170	2.5494	15.934
502-274 1010	Wheat Flour	0.51260	2.5367	15.854
502-274 1010	Wheat Flour	0.50370	2.5394	15.871
502-274 1010	Wheat Flour	0.50170	2.5290	15.806
502-274 1010	Wheat Flour	0.50230	2.5360	15.850
502-274 1010	Wheat Flour	0.50300	2.5329	15.830
502-274 1010	Wheat Flour	0.51440	2.5426	15.891
502-274 1010	Wheat Flour	0.50210	2.5366	15.854
502-274 1010	Wheat Flour	0.50310	2.5378	15.861
502-274 1010	Wheat Flour	0.50180	2.5306	15.816

Element	Average	Std. Deviation	RSD	Count
Mass	0.50564	0.005	1.006	10
Nitrogen %	2.5371	0.00591	0.233	10
Protein %	15.857	0.0369	0.233	10

- Easy-of-use operating software with results management and data export (LIMS)
- Wide dynamic range linear calibration with drift correction

Example Ham

TRU MAC

Ham Data

Name	Comments	Mass	Nitrogen %	Protein %	Method
131755	Ham	1.98300	4.1351	25.844	EDTA CAL (8-28-09) 1350C
131755	Ham	2.04480	4.1333	25.833	EDTA CAL (8-28-09) 1350C
131755	Ham	2.18440	4.1360	25.850	EDTA CAL (8-28-09) 1350C
131755	Ham	2.12180	4.1238	25.773	EDTA CAL (8-28-09) 1350C
131755	Ham	2.16780	4.1794	26.121	EDTA CAL (8-28-09) 1350C
131755	Ham	2.21370	4.1312	25.820	EDTA CAL (8-28-09) 1350C
131755	Ham	2.02260	4.1227	25.767	EDTA CAL (8-28-09) 1350C
131755	Ham	1.95390	4.1461	25.913	EDTA CAL (8-28-09) 1350C
131755	Ham	2.22070	4.1827	26.142	EDTA CAL (8-28-09) 1350C
131755	Ham	2.11600	4.1324	25.827	EDTA CAL (8-28-09) 1350C

Element	Average	Std. Deviation	RSD	Count
Mass	2.10287	0.10	4.592	10
Nitrogen %	4.1423	0.02145	0.518	10
Protein %	25.889	0.1341	0.518	10

Example Meat

Turkey Data

Name	Comments	Mass	Nitrogen %	Protein %
131756	Turkey	1.04190	4.2318	26.449
131756	Turkey	1.04950	4.2162	26.351
131756	Turkey	1.04560	4.1914	26.196
131756	Turkey	1.01480	4.2385	26.491
131756	Turkey	1.04090	4.2423	26.514
131756	Turkey	1.06230	4.2205	26.378
131756	Turkey	1.08060	4.2241	26.400
131756	Turkey	1.10820	4.2362	26.476
131756	Turkey	1.07540	4.1897	26.186
131756	Turkey	1.07850	4.1931	26.207

Element	Average	Std. Deviation	RSD	Count
Mass	1.05977	0.03	2.513	10
Nitrogen %	4.2184	0.02030	0.481	10
Protein %	26.365	0.1269	0.481	10

Example Starch

Name	Gewicht in g	Stickstoff in %	Proteinfaktor	Protein in %	Datum
Stärke A	~1 g	0,0427	6,25	0,2669	01.06.2013
Stärke A	~1 g	0,0424	6,25	0,2651	01.06.2013
Stärke A	~1 g	0,0421	6,25	0,2628	01.06.2013
Stärke A	~1 g	0,0421	6,25	0,2631	01.06.2013
Stärke A	~1 g	0,0421	6,25	0,2628	01.06.2013
Stärke A	~1 g	0,0427	6,25	0,2670	01.06.2013
Stärke A	~1 g	0,0422	6,25	0,2638	01.06.2013
Stärke A	~1 g	0,0419	6,25	0,2619	01.06.2013
Stärke A	~1 g	0,0427	6,25	0,2669	01.06.2013
Stärke A	~1 g	0,0427	6,25	0,2668	01.06.2013
Mittelwert		**0,0424**		**0,2647**	
Abweichung (+-)		**0,00033**		**0,00206**	
RSD		**0,78**		**0,78**	

Example homogenous chemical

TRU MAC

Caffeine Data

Name	Mass	Description	Carbon %	Nitrogen %	Analysis Date
502-205 1010	0.1522	Caffeine @ 49.48%C, 28.85%N	49.311	28.816	1/7/2011 12:21:49 PM
502-205 1010	0.1516	Caffeine @ 49.48%C, 28.85%N	49.354	28.848	1/7/2011 12:28:36 PM
502-205 1010	0.1524	Caffeine @ 49.48%C, 28.85%N	49.339	28.871	1/7/2011 12:35:24 PM
502-205 1010	0.1502	Caffeine @ 49.48%C, 28.85%N	49.238	28.810	1/7/2011 12:42:12 PM
502-205 1010	0.1547	Caffeine @ 49.48%C, 28.85%N	49.325	28.862	1/7/2011 12:49:00 PM
502-205 1010	0.1516	Caffeine @ 49.48%C, 28.85%N	49.341	28.875	1/7/2011 12:55:48 PM
502-205 1010	0.1527	Caffeine @ 49.48%C, 28.85%N	49.264	28.852	1/7/2011 1:02:36 PM
502-205 1010	0.1537	Caffeine @ 49.48%C, 28.85%N	49.322	28.902	1/7/2011 1:09:24 PM
502-205 1010	0.1538	Caffeine @ 49.48%C, 28.85%N	49.503	28.962	1/7/2011 1:16:11 PM
502-205 1010	0.1527	Caffeine @ 49.48%C, 28.85%N	49.264	28.859	1/7/2011 1:22:59 PM

Element	Average	Std. Deviation	RSD	Count
Mass	0.1526	0.001	0.847	10
Carbon %	49.326	0.0732	0.148	10
Nitrogen %	28.866	0.0433	0.150	10

FP628 Series Software

628 SERIES

Barley 502-277

Name	Mass	Description	Nitrogen %	Analysis Date
Barley 502-277 1008	0.2542	@44.72%C, 1.79%N	1.7778	5/19/2011 3:21:20 PM
Barley 502-277 1008	0.2537	@44.72%C, 1.79%N	1.7790	5/19/2011 3:25:04 PM
Barley 502-277 1008	0.2519	@44.72%C, 1.79%N	1.7905	5/19/2011 3:28:49 PM
Barley 502-277 1008	0.2533	@44.72%C, 1.79%N	1.7807	5/19/2011 3:32:33 PM
Barley 502-277 1008	0.2521	@44.72%C, 1.79%N	1.7889	5/19/2011 3:36:19 PM

Element	Average	Std. Deviation	RSD	Count
Mass	0.2530	0.001	0.397	5
Nitrogen %	1.7834	0.00589	0.330	5

FP628 Data

628 SERIES

LECO Corn Flour 502-563

Name	Mass	Description	Nitrogen %	Analysis Date
Corn Flour 501-563 1011	0.2529	@1.46%N	1.4564	5/19/2011 3:40:04 PM
Corn Flour 501-563 1011	0.2545	@1.46%N	1.4569	5/19/2011 3:43:50 PM
Corn Flour 501-563 1011	0.2513	@1.46%N	1.4574	5/19/2011 3:47:35 PM
Corn Flour 501-563 1011	0.2505	@1.46%N	1.4544	5/19/2011 3:51:20 PM
Corn Flour 501-563 1011	0.2530	@1.46%N	1.4499	5/19/2011 3:55:04 PM

Element	Average	Std. Deviation	RSD	Count
Mass	0.2524	0.002	0.621	5
Nitrogen %	1.4550	0.00305	0.210	5

FP628 Data

628 SERIES

Glycine 0.1% N Solution

Name	Mass	Description	Nitrogen %	Analysis Date
Glycine 0.1%	0.2937	@0.172%C, 0.100%N	0.09916	5/24/2011 12:00:24 PM
Glycine 0.1%	0.3198	@0.172%C, 0.100%N	0.09782	5/24/2011 12:04:10 PM
Glycine 0.1%	0.3088	@0.172%C, 0.100%N	0.09772	5/24/2011 12:07:55 PM
Glycine 0.1%	0.3237	@0.172%C, 0.100%N	0.09677	5/24/2011 12:11:40 PM
Glycine 0.1%	0.3203	@0.172%C, 0.100%N	0.10076	5/24/2011 12:15:25 PM

Element	Average	Std. Deviation	RSD	Count
Mass	0.3133	0.01	3.922	5
Nitrogen %	0.09845	0.001549	1.574	5

Example Argon / Helium

	Teststärke mit Helium			Teststärke mit Argon		
	Einwaage	N in %	Protein in %	Einwaage	N in %	Protein in %
Eichstärke	~ca 2 g	0,0340	0,2126	~ca 2 g	0,0351	0,2192
Eichstärke	~ca 2 g	0,0345	0,2157	~ca 2 g	0,0348	0,2174
Eichstärke	~ca 2 g	0,0350	0,2184	~ca 2 g	0,0334	0,2085
Eichstärke	~ca 2 g	0,0347	0,2166	~ca 2 g	0,0343	0,2141
Eichstärke	~ca 2 g	0,0347	0,2166	~ca 2 g	0,0353	0,2204
Eichstärke	~ca 2 g	0,0343	0,2143	~ca 2 g	0,0349	0,2183
Eichstärke	~ca 2 g	0,0342	0,2137	~ca 2 g	0,0340	0,2124
Eichstärke	~ca 2 g	0,0348	0,2174	~ca 2 g	0,0342	0,2137
Eichstärke	~ca 2 g	0,0342	0,2137	~ca 2 g	0,0338	0,2109
Eichstärke	~ca 2 g	0,0355	0,2217	~ca 2 g	0,0351	0,2193
Eichstärke	~ca 2 g	0,0342	0,2138	~ca 2 g	0,0350	0,2185
Mittelwert:		**0,0345**			**0,0345**	
SA		**0,00043**			**0,00063**	
RSA		**1,24**			**1,84**	

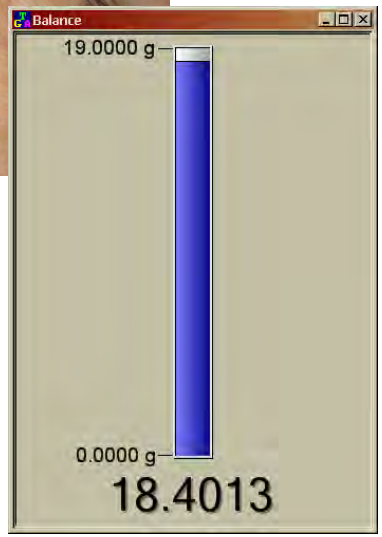
and ….

TGA 701: Moisture and Ash

Samples logged into software and empty crucibles loaded into TGA701 carousel for taring

Green button on TGA701 is pressed to begin the tare

TGA 701



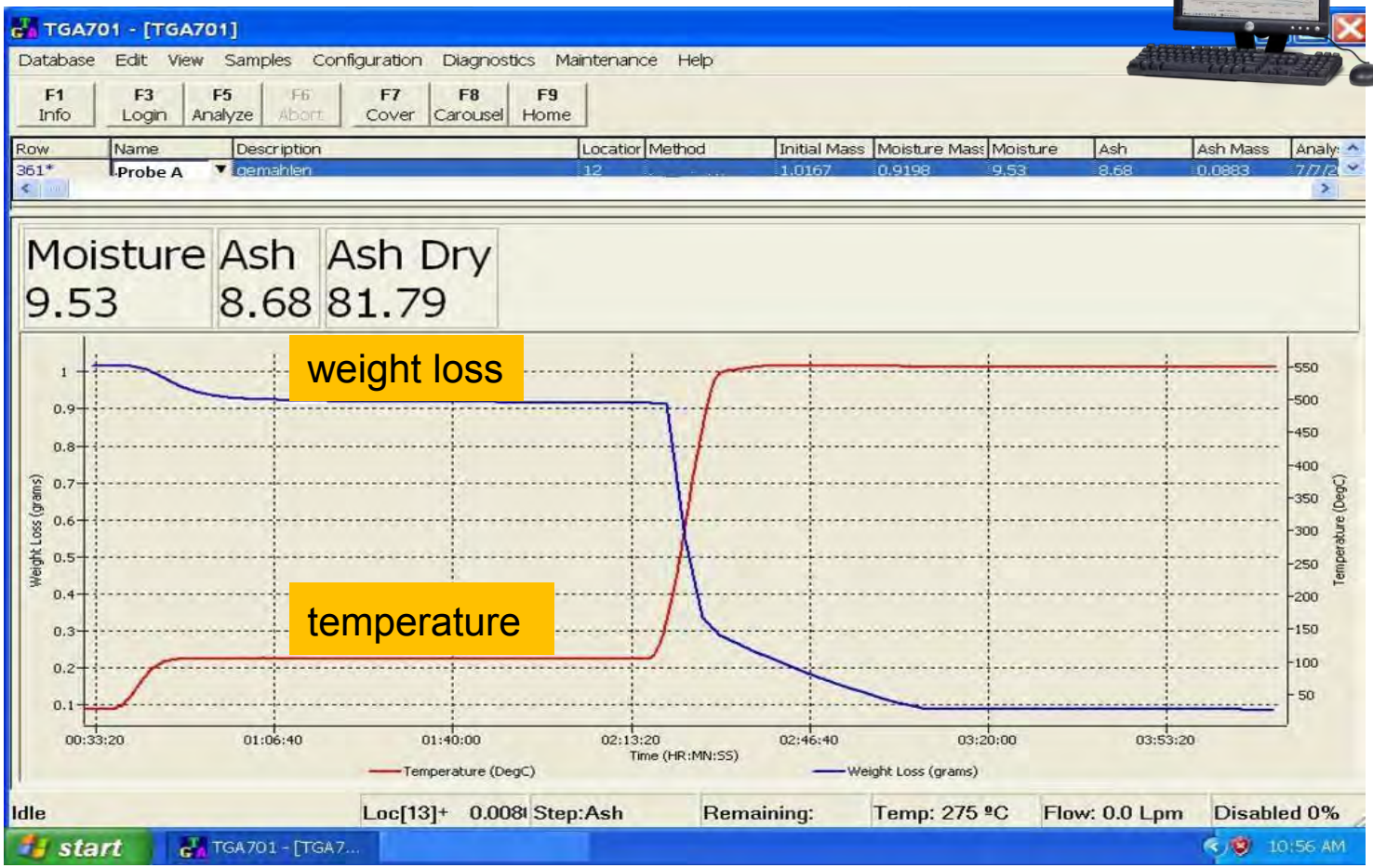
Sample placed into tared crucibles

Operator may use live balance display in software as a reference

Green button pressed and the analysis run begins

Leco®
Delivering the Right Results

TGA-701



The **TGA-701** measures the weight loss of organic, inorganic, and synthetic material as a function of temperature in a controlled environment.

The instrument consists of a computer and a multiple sample furnace that allows up to 19 samples to be analyzed simultaneously

VERENIGING VOOR NUTRIENTEN ANALYSE

Hartelijk dank voor uw belangstelling!

Delivering the Right Results