Elemental Analyzer

Cell composition

 $CH_xO_yN_z$

Dry weight vs. wet weight 70% of the composition is water

Dry weight consists of:

Element	E. coli	<u>Yeast</u>
	 00/	- 00/
С	50%	50%
0	20%	34%
N	14%	8%
Н	8%	6%
Р	3%	1%
S	1%	<1%
K	1%	<1%
Na	1%	<1%
Others	<1%	<1%

<u>Element</u>	% dry wgt	<u>Source</u>
Carbon	50	organic compounds or CO ₂
Oxygen	20	H ₂ O, organic compounds, CO ₂ , and O ₂
Nitrogen	14	NH ₃ , NO ₃ , organic compounds, N ₂
Hydrogen	8	H ₂ O, organic compounds, H ₂
Phosphorus	3	inorganic phosphates (PO ₄)
Sulfur	1	SO ₄ , H ₂ S, S°, organic sulfur compounds
Potassium	1	Potassium salts
Magnesium	0.5	Magnesium salts
Calcium	0.5	Calcium salts
Iron	0.2	Iron salts

Example

$$C_6H_{12}O_6 + a O_2 + b NH_3 \rightarrow c C_{4.4}H_{7.3}O_{1.2}N_{0.86} + dH_2O + eCO_2$$

- 2/3 of the glucose C goes to biomass
- What are the stoichiometric coefficients, and $Y_{x/s}$, $Y_{x/O2}$?

$$MW_{glucose} = 180$$

 $MW_{cell} = 89.62$
 $MW_{oxygen} = 32$
 $MW_{ammonia} = 17$

Generalized growth reaction

$$C_6H_{12}O_6 + a NH_3 + b O_2 \longrightarrow \alpha CH_{1.8}O_0 N_{0.2} + \beta CH_xO_yN_z + \gamma CO_2 + \delta H_2O_2$$

- Normalized to 1 mole of carbon source compound
- Where a, b, α , β , γ , δ , x, y, z depend on the type of cell involved.
- a, b, α , β , γ , δ , are stoichiometric coefficients
- When little info is available about cell composition, use an approximated cell composition of CH_{1.8}O_{0.5}N_{0.2}
- This yields a MW of a cell ~ 24.6
- Ash content contributed by other inorganics ~ 5 -10 %
- Actual MW $\approx \frac{24.6}{0.95}$

Chemical Methods

Elemental Methods: Carbon and Hydrogen Analysis

- Combustion Analysis:
 - Typically used to determine C and H content of <u>pure compounds</u>
 - Based on burning the sample in the presence of excess oxygen, converting all C, H, N and S into volatile oxides:

$$C_nH_mO_oN_pS_q \xrightarrow{O_2/heat} n CO_2 + m/2 H_2O + p O_2 + q SO_2$$

• To get at O content, method may be modified by using F₂ in place of O₂ as the oxidizing agent:

$$C_nH_mO_oN_pS_q \xrightarrow{F_2/heat} n CF_4 + m HF + o OF_2 + p NF_3 + q SF_4$$

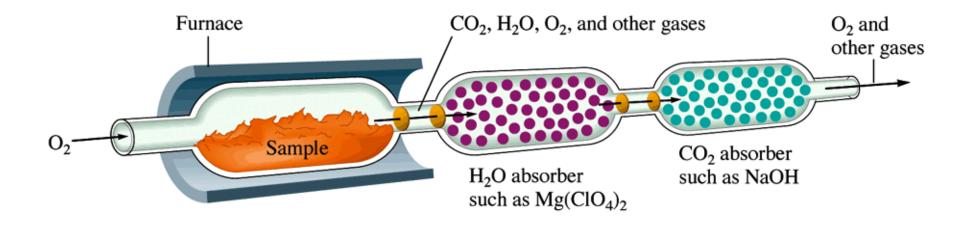
• <u>Problem:</u> much more hazardous, difficult and expensive to do than normal combustion analysis with O₂. Best performed by robots or automated systems.

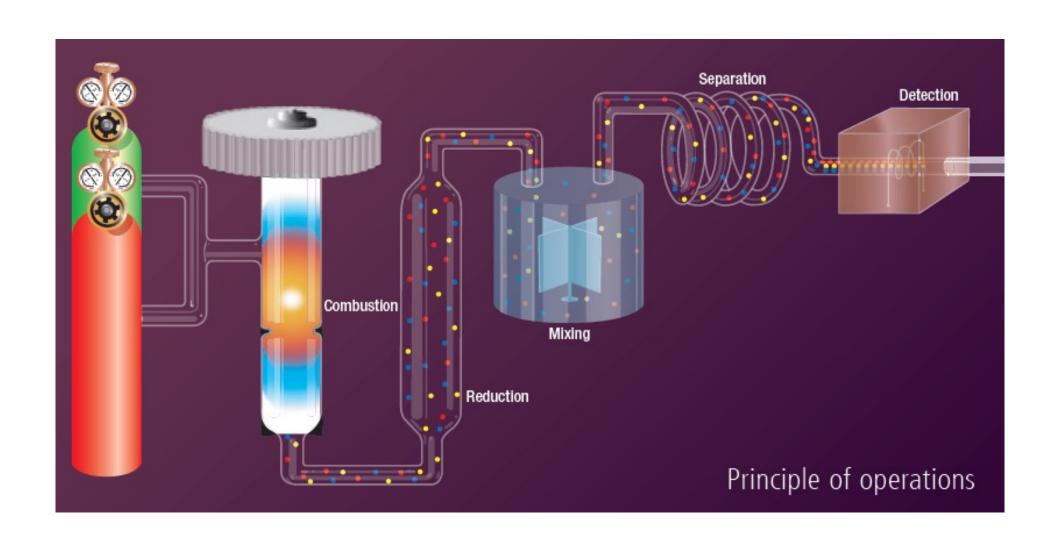
Elemental Analysis (EA)

- Weight percentages of C, H, N, S done by combustion in O₂

- Gas chromatographic analysis of CO₂, H₂O and N₂, SO₂

- Oxygen as CO





C/H/N/S Elemental Analysis

The sample and tin capsule react with oxygen and combust at temperatures of 1700-1800 °C and the sample is broken down into it's elemental components, N₂, CO₂, H₂O and SO₂.

High performance copper wires absorb the excess oxygen not used for sample combustion.

Oxygen Analysis

- A pyrolysis reactor which is filled with a special nickelized carbon wool contact material heated to 1080°C,
- A trap for acidic gases formed by the pyrolysis

Samples are dropped automatically into the pyrolysis reactor, break down and the released oxygen reacts with the nickel carbon wool to form CO (2C + O_2 = 2 CO) and N_2 , if present in the sample. CO peak is detected by the TCD.

Elemental Analyzer Core Operations

- Combustion
 - Sample combusts in furnace at 950 deg C to 1200 deg C
 - Sample drops into crucible
 - Jet injection of oxygen via lance right over/at sample
 - Creates turbulent flow around sample
 - like blowing on a fire



Elemental Analyzer Core Operations

Gas Separation

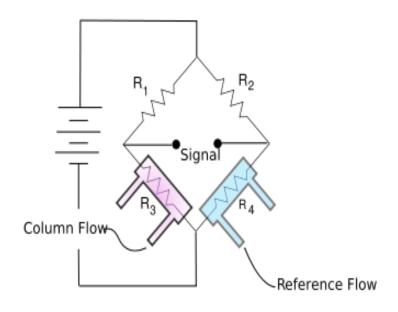
- Helium carrier gas pushes combustion gases through analyzer. Argon is alternate gas.
- C, H and S combustion gases are trapped in separate columns, then sequentially released (aka "purge and trap").
- N₂ gas is not trapped. It flows straight thru columns.



Core Operations

Gas Detection

- TCD Thermal Conductivity Detector.
 Combustion gases and reference gas have different thermal conductivities.
- R3 and R4 are thermistors (resistors whose resistance changes with temperature).
- TCD creates voltage signal which is integrated over time and calibrated to elemental mass.
- The voltage signal is created as follows:
 - Electric current in R3 and R4 thermisters increases their temperatures relative to TCD metal mass surrounding path of gas flow.
 - R3 thermister is cooled by thermal conductivity of column gas. R4 thermister is cooled by thermal conductivity of reference gas.
 - R3 vs R4 temperature difference creates difference in thermister resistance which creates voltage signal.



Chemistry

Combustion

R-N + O₂ ---->
$$N_2$$
 + NO_x + O₂ + CO + CO₂ + CH₄ + X⁻ + SOx + H₂O

Post Combustion

$$CO + CH_4 --- \frac{CuO \& Pt}{} --> CO_2 + H_2O$$

Reduction

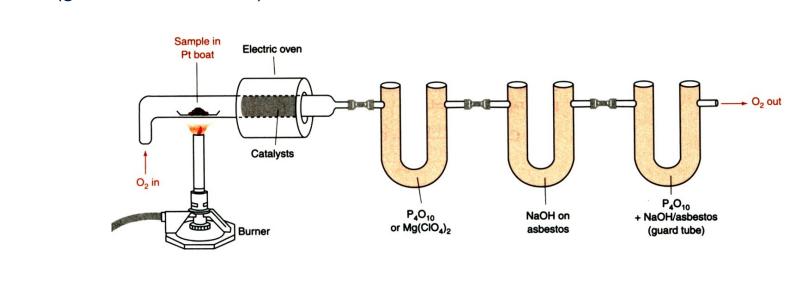
$$NO_x+O_2+CO_2-\frac{tungsten}{---} N_2+WO_3+CO$$
 (trace)
CO + CuO -----> CO₂

More Chemistry

- Removal of other gases
 - X⁻ via reaction with silver wool
 - H₂O via condensation, membrane dryer
 - Trace SO₂ via reaction with tungsten
- Nitrogen
 - detected by TCD
- Carbon Dioxide
 - rapid N "fades" into carrier gas
 - Max and Macro separated on purge and trap column, then released and detected by TCD.

Chemical Methods

- II. Methods for Quantitation of Gases in Combustion Analysis *Gravimetric Analysis*
 - Pass gases through series of cartridges that selectively absorb CO₂, H₂O, etc. (gas-solid extraction)





- Weigh the solid cartridges before and after burning the sample.
- The difference in weight is related to the amount of gas produced and the composition of the original sample.
 - P₄O₁₀ can be used to absorb H₂O
 - Ascarite (NaOH coated non-fibrous silicate) can be used to absorb CO₂

Chemical Methods

- II. Methods for Quantitation of Gases in Combustion Analysis *Gravimetric Analysis*
 - Advantages of Gravimetric Analysis
 - Method is easy to perform and inexpensive
 - High accuracy and precision is possible
 - Sample requirement is in the μg/mg range.
 - Disadvantages of Gravimetric Analysis
 - Need complete combustion for good accuracy
 - Must consider selectivity of the adsorption cartridges



Chemical Methods

- III. Methods for Quantitation of Gases in Combustion Analysis *Gas Chromatography*
 - Collect gasses produced by combustion by using a cold trap or cartridge.
 - Inject the mixture of gases onto a GC system to separate and quantitate individual components (e.g., CO₂, H₂O, NO₂, SO₂)

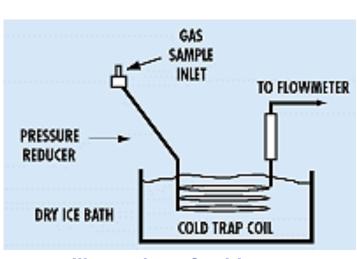
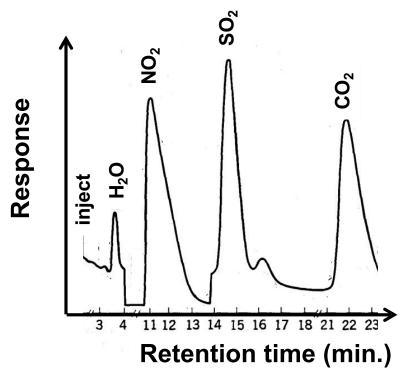


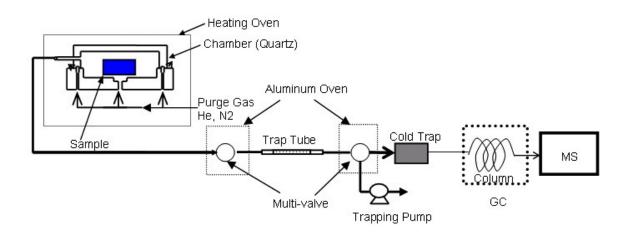
Illustration of cold trap



Typical GC chromatogram

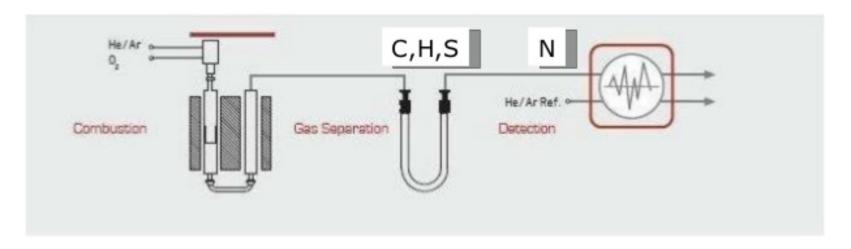
Chemical Methods

- III. Methods for Quantitation of Gases in Combustion Analysis *Gas Chromatography*
 - Commonly used in professional labs for C/H analysis
 - Advantages of GC
 - Easier to perform than gravimetric analysis
 - Less subject to interferences from other element.
 - Sample requirement lower than gravimetric method (ng-μg)
 - Can do multiple elements in a single run
 - Disadvantages of GC
 - More expensive than gravimetric analysis





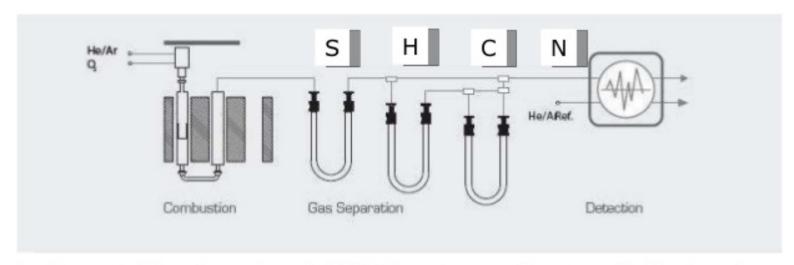
Temperature programmed desorption with single-column technology



The "TPD" technology is a very straight forward method to separate the gaseous compounds by complete adsorption and subsequent element specific desorption. It guarantees baseline separation of CHNS under all conditions.



Purge and Trap with multi-column chromatography



In the vario EL cube and vario MACRO cube a multi-column technology is realized to separate even larger sample sizes. The gaseous compounds are adsorbed at element specific columns and one after the other released by a temperature supported desorption. All elements are analyzed simultaneously from a single sample.

Percentage Composition

 $C_xH_yO_z$ (9.83 mg) + excess $O_2 ---- \rightarrow x CO_2$ (23.26 mg) + y/2 H_2O (9.52 mg)

millimoles of CO_2 = 23.26 mg/ 44.01mg/mmol = 0.5285 mmoles of CO_2 mmoles of CO_2 = mmoles of C in original sample (0.5285 mmoles of C)(12.01mg/mmol C) = 6.35 mg of C in original sample

 $C_xH_yO_z$ (9.83 mg) + excess $O_2 \longrightarrow x$ CO_2 (23.26 mg) + y/2 H_2O (9.52 mg) mmoles of H_2O = 9.52 mg/ 18.02 mg/mmol = 0.528 mmoles of H_2O mmoles of H_2O = 1/2 mmoles of H in original sample (0.528 mmoles of H)(2)(1.008mg/mmol H) = 1.06 mg of H in original sample 765u

Weight Percentage Composition

```
C_xH_yO_z (9.83 mg) + excess O_2 \longrightarrow x CO_2 (23.26 mg) + y/2 H_2O (9.52 mg) %C = 6.35 mg/9.83 mg x 100 = 64.6% %H = 1.06 mg/9.83 mg x 100 = 10.8% %O = 100-(64.6 + 10.8) = 24.6%
```

Calculation of Empirical Formula

assume for example a 100g sample

64.6% of C: 64.6 g/12.01 g/mol = 5.38 moles of C

10.8% of H: 10.8 g/1.008 g/mol = 10.7 moles of H

24.6% of O: 24.6 g/16.0 g/mol = 1.54 moles of O

Thus: C5.38 H10.7 O1.54

converting to simplest ratio:

C5.38/1.54; H10.7/1.54; O1.54/1.54

 $C_{3.50} H_{7.00} O_{1.00} = C_7 H_{14} O_2$

What are possible structures?

Typical applications

Organics and Pharmaceuticals – organophosphorous and steroids

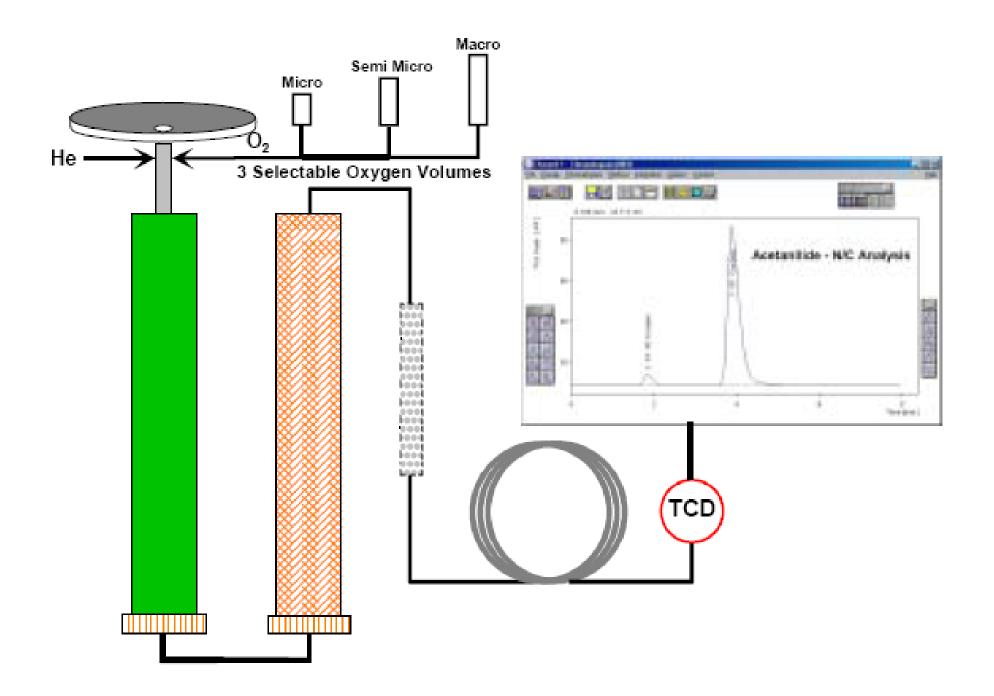
Environmental - fuels, oils, the composition of material retained by membranes

Polymers - determining elemental composition of polymers, copolymers, and blends.

Refractories - Nitrides, graphite fibers, and ceramics

Volatile/Air Sensitive Samples - Volatile and air sensitive materials

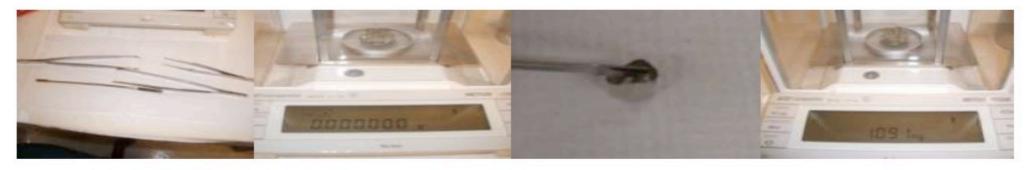
Other Application Areas: Plastics, petrochemicals, agriculture, food, and pyrotechnical compounds.



Sample Preparation

- The sample (1-3 mg and ≤ 10 mg)
- 2. Weighed in a tin capsule and weighed twice: once after filling the tin capsule and again after folding.
- 3. Don't use it if the weight is far less than original after folding. There may have been a break in the tin capsule.

Step by step sample and tin capsule preparation



1. clean all tools with methanol

place 4 tins in balance and re-zero

3.take one tin out and add sample using clean spatula

4. record the sample weight



press air out of the tin and close the capsule using 2 tweezers



6. twist tin as Z shape from side to side using two tweezers



fold tin into 1/3 of original width (Z twist)



8. fold 1/3 of tin from top



9. fold one more time

10. bend the tin to avoid getting stuck in auto sampler



 record the weight should be close to first measure (step 4)

12. repeat step 1 to 11 for rest of samples

- The solid sample is placed in autosampler
- The tin capsule containing sample is burned in a pure oxygen environment (99.996%).

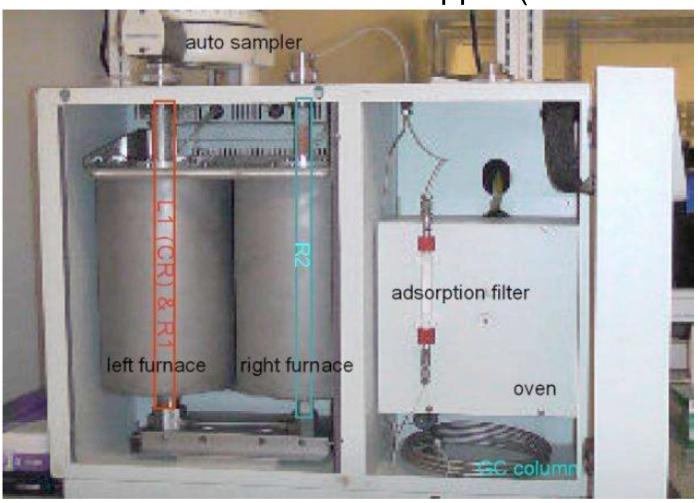


Two furnaces (left and right)

 The steel column is inside the left furnace and contains the crucible (CR or L1) and reactors (R1; Oxidation Reactor filled with oxidation catalyst).

A quartz column filled with reduced copper (R2: reduction)

reactor).



The materials used in the reactors and adsorption filter

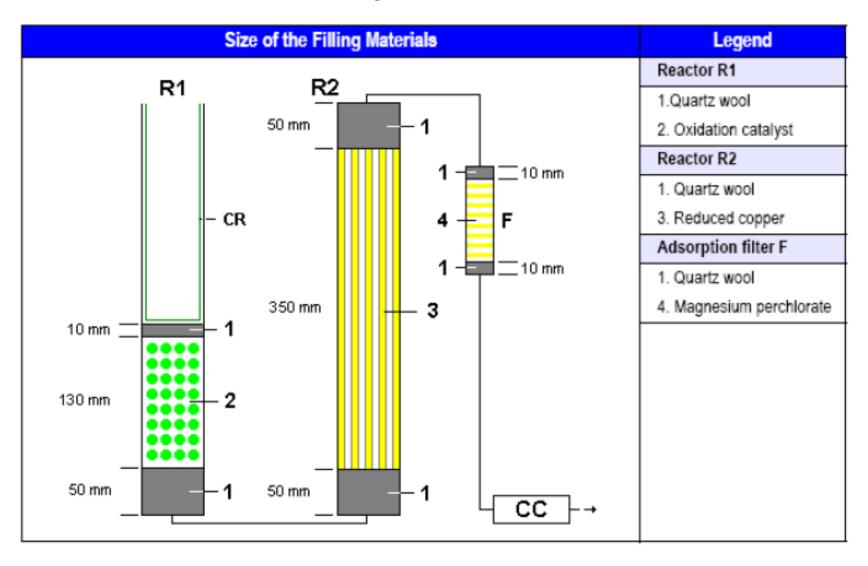


Figure 2 Materials used in both reactors and adsorption filter.

The left reactor (L1 & R1)

- A steel column is surrounded by a furnace operated at 900°C

- The combustion converts carbon to carbon dioxide, nitrogen to free gas or some oxides and hydrogen to water.

$$C + O_2 \longrightarrow CO_2$$

 $2H + \frac{1}{2}O_2 \longrightarrow H_2O$
 $4N + x O_2 \longrightarrow N_2 + 2 NO_x$

R2: right reactor

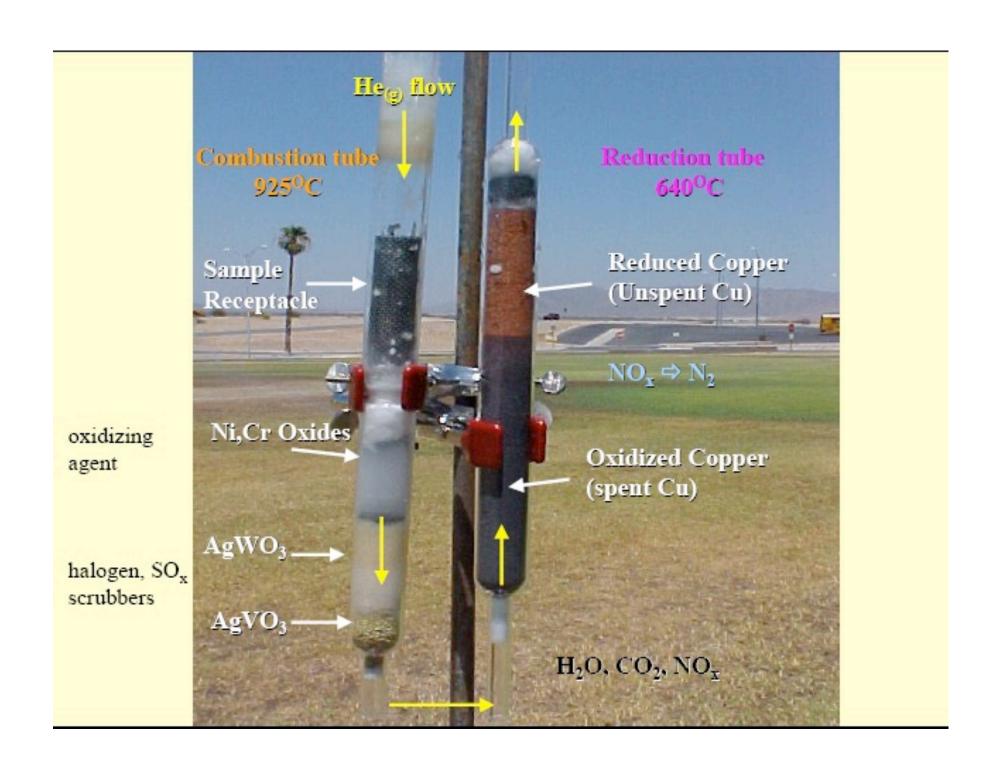
- Filled with copper that reduces the nitrogen oxides to nitrogen and removes the excess oxygen at 680°C.

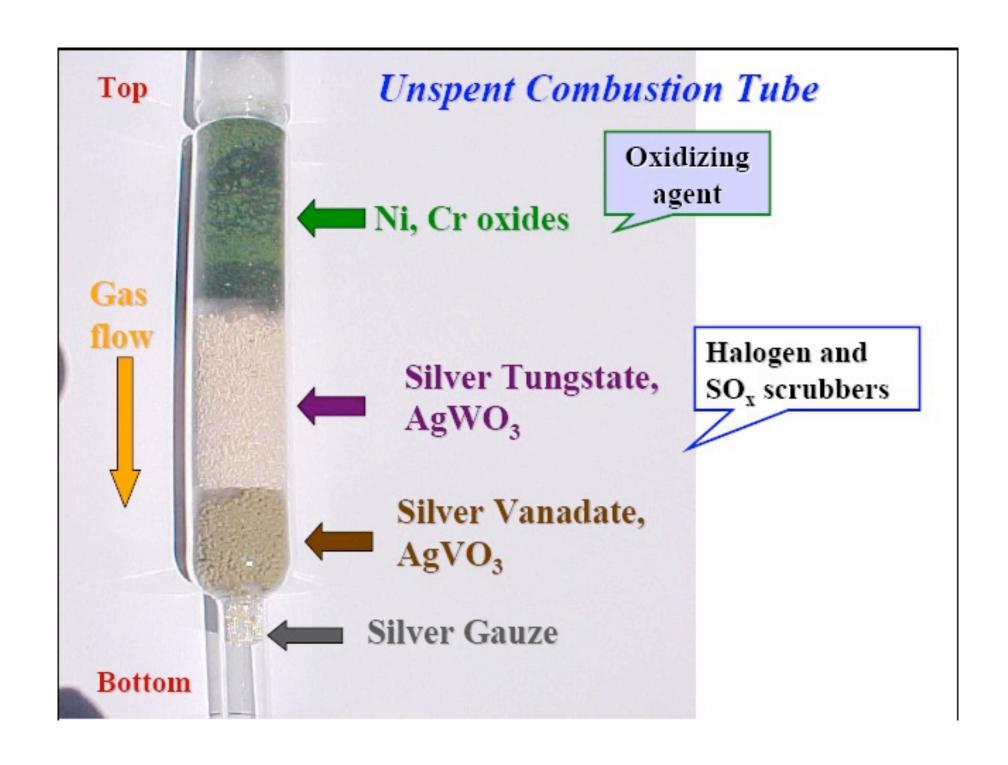
$$NO_x \longrightarrow N_2 + CuO$$

$$O_2$$
 + Cu \longrightarrow CuO

Absorption filter

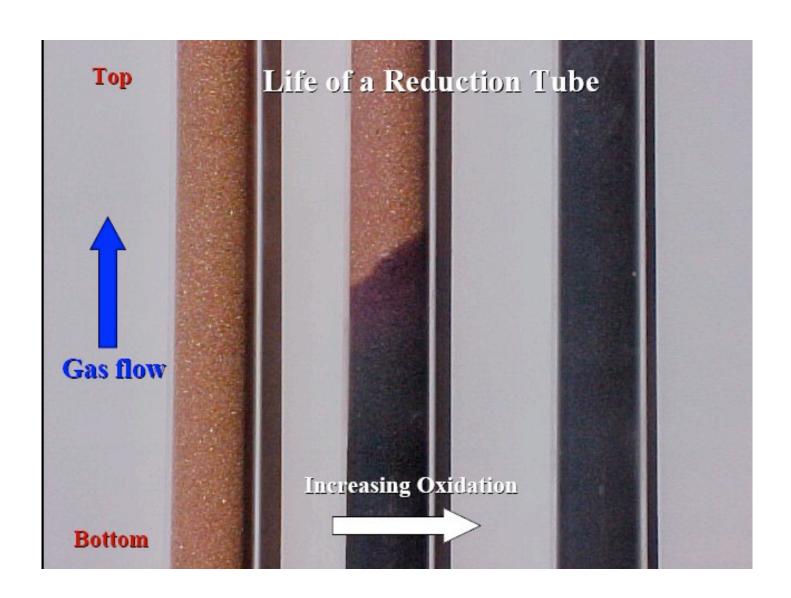
- A magnesium perchlorate trap removes water before CO₂ and N₂ gases go into a gas chromatograph (GC)
- Column at room temperature.





Reduction Tube

- Filled with zero valent (elemental) copper (neither nickel nor zinc)

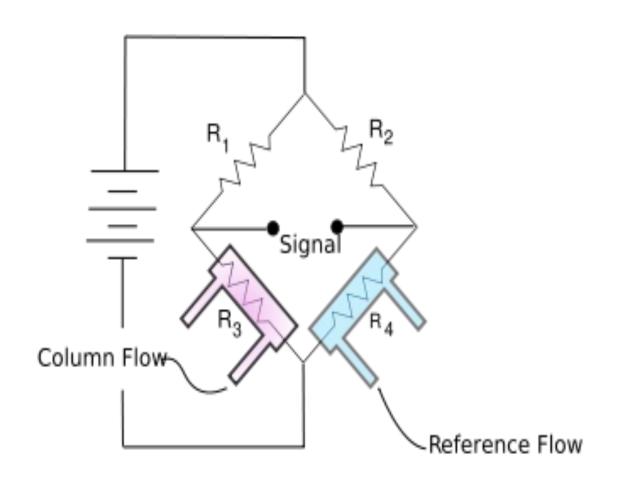


Gas Separation

- Helium carrier gas pushes combustion gases through analyzer. Argon is an alternate gas.
- C, H and S combustion gases are trapped in separate columns, then sequentially released ("purge and trap").
- **№** N₂ gas is not trapped. It flows straight through columns.

Gas Detection

TCD – Thermal Conductivity Detector (Combustion gases and reference gas have different thermal conductivities)



standards

The standards selected cover a large range of concentration from 4.84 to 46.65 % for nitrogen and from 20 to 71.09 % for carbon.

Table 1 – Standards for CHN determination

Standard	N %	C %	Н%
Acetanilide	10.36	71.09	6.71
CEDFNI	20.14	51.79	5.07
Urea	46.65	20.00	6.71
Atropine	4.84	70.56	8.01

Note: CEDFNI: Cyclohexanone-2,4-dinitrophenylhydrazone

Organic Analytical Standards

Acetanilide

C=71.09% H=6.71% N=10.36%

O=11.84%

Atropine

C=70.56% H=8.01% N=4.84%

O=16.59%

Benzoic Acid

C=68.85% H=4.95% O=26.20%

Cyclohexanone 2-4 dinitrophenyl hydrazone

C=51.79% H=5.07% N=20.14%

O=23.00%

Sulfanilamide

C=41.84% H=4.68% N=16.27%

O=18 58% S=18 62%

BBOT

2,5-Bis (5-tert-butyl-benzoxazol-2yl) thiophene

C=72 53% H=6 09% N=6 51%

O=7.43% S=7.44%

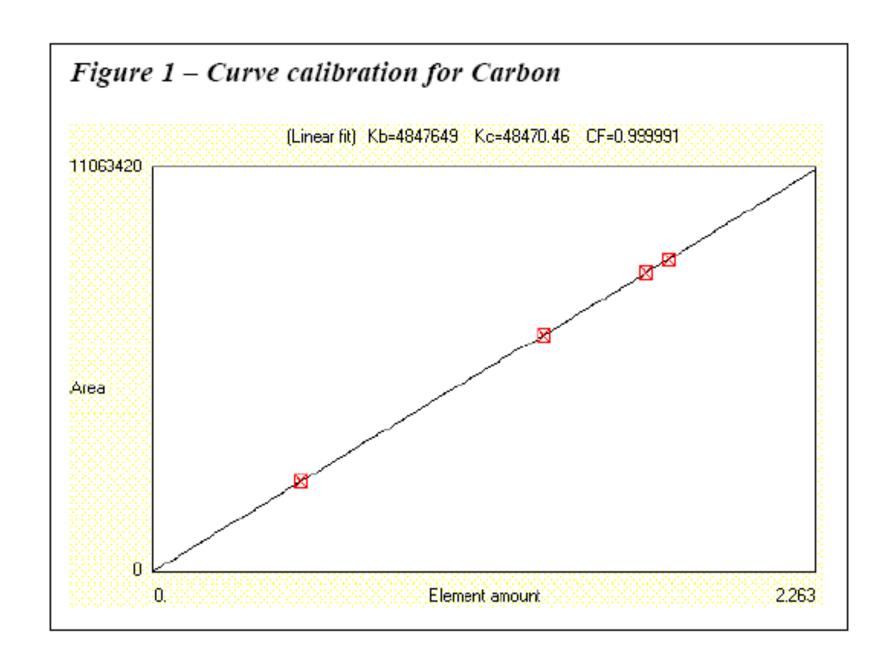
Methionine

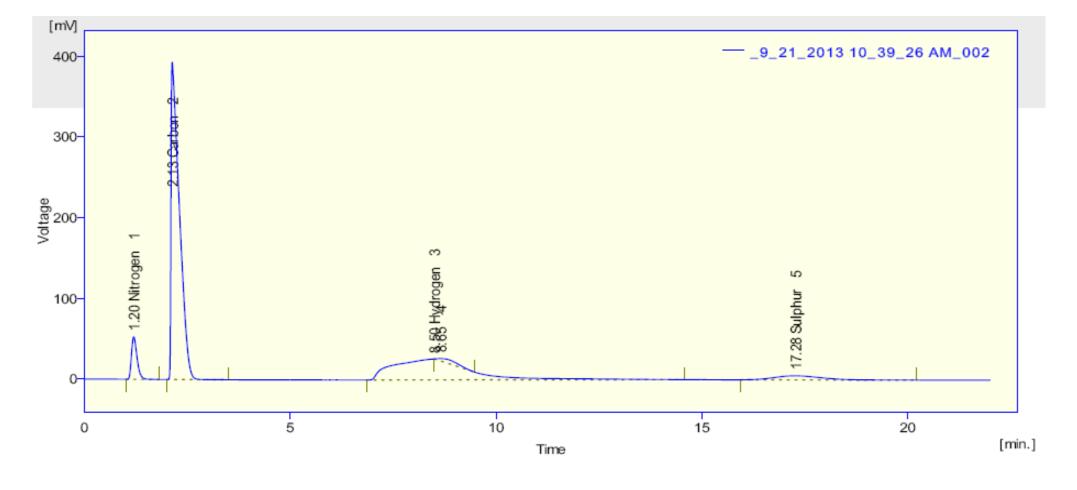
N=9.39%

C=40.25%



Calibration curve for the carbon element





Result Table (ESTD - _9_21_2013 10_39_26 AM_002)

	Reten. Time	Response	Weight	Weight	Peak	Element	Carbon Response
	[min]		[mg]	[%]	Туре	Name	Ratio
1	1.197	494.011	0.179	1.93	Refer	Nitrogen	0.089
2	2.133	5530.060	0.825	8.88	Refer	Carbon	1.000
3	8.497	3324.355	0.164	1.76	Refer	Hydrogen	0.601
4	8.653	150.575	0.000	0.00			???
5	17.277	396.706	0.124	1.34	Refer	Sulphur	0.072
	Total		9.290	13.91			
						-	

Sample Weight [mg]: 9.2900

$TABLE \ 1-List \ of \ analytical \ troubleshooting$

Problem	Diagnosis	Remedy
High N blank	Presence of leak.	Check that Helium and Oxygen lines are leak free
		if not eliminate possible leaks.
		5 4 5 4 5 4
	Oxygen line or cylinder contaminated.	Purge the line, or replace the cylinder.
	Autosampler not purged	Check that the flow is correct (100 ml/min for 1
	(see page 4)	drum, 150-200 ml/min for more drums)
High constant N blank	Oxygen cylinder contaminated.	Replace the Oxygen cylinder.
in several sequential		
runs	Presence of leak in the autosampler.	Identify leaks and remove them.
_	Oxygen line contaminated.	Purge the line 10-20 minutes. Repeat blank run.
values. Increasing N blank	Copper exhausted (see page 13)	Replace the reactor.
value.	Copper exhausted (see page 15)	Replace the feactor.
Tailing on N peak	Bad combustion (see page 5, 10)	Decrease weight of sample, increase oxygen
	, 10 ,	quantity, clean ashes.
High C blank.	Tin capsules contaminated.	Check capsule box, tweezers, work bench must be
		clean.
	Memory effect to bad combustion.	Remove ashes and/or analyze lower amount of
Tailing or split on C	Too many ashes.	sample. Check ashes and remove them.
peak.	100 many asnes.	Check asnes and remove them.
реак.	Bad combustion (see page 5)	Decrease weight of sample, increase oxygen
	Zue comounten (see puge s)	quantity.
	MAS200 cover position not correct.	Check the MAS200 cover position.
	(see page 5)	
Bad separation between	High Nitrogen blank value.	Check the Nitrogen blank. Repeat the analysis.
N and C.	Copper exhausted (see page 13)	Replace the reactor.
Peak between N and C.	Oxygen line contaminated	Exclude autosampler and check the oxygen blank.
remi servecii i min c.	onygen mie condiminates	Energie and energia and energia and only gen oranic.
	Inadequate Oxygen purity.	Exclude autosampler and check the oxygen blank.
		Increase the Helium Reference flow to 150-200
	incomplete (see page 4).	ml/min.
	Sample delay not correct (see page 9)	Increase or decrease the sample delay time.
Negative peak between		Regulate both pressures to the same value 250 kPa.
N and C	the same in CHN/O and CHNS/O	and the second pressures to the state that are all and
	configurations (see page 3)	
Split on H peak.	The tube connecting reactor and GC	Cut off the clogged tube portion.
	column is clogged.	
Retention time very	Presence of leaks or obstructions in	Perform a leak test or reach and remove the
delated respect to the	the pneumatic circuit.	obstruction.
typical chromatogram.	Anhydrone exhausted (see page 14)	Change Aphydrone
	Amiyarone exhausted (see page 14)	Change Anhydrone.

TABLE 2 - Analytical Troubleshooting related to sample nature.

Problem	Diagnosis	Remedy
Sulfur peak:	Incomplete Sulphur conversion	Add about 10 mg of vanadium pentoxide to the tin
tailing or split	(for example in soil, leaves,	container before weighing the sample and mix them.
(see page 15)	plants, samples with inorganic	
	sulphur).	
Hydrogen peak: Split	Presence of fluorine in the sample	For CHNS/NCS use Silver capsule (P/N 240.054.05)
(sometimes also ghost		and weigh 0.2-0.4 mg of sample).
peak on Carbon peak)		For CHN fll the reactor with a specific absorber mix
(see page 16)		before the catalyst.

Effect of the Helium and Oxygen pressure

The nominal pressure of gas supplies: 250 kPa for Helium and 250 or 300 kPa for Oxygen

Trouble: if the Helium and Oxygen pressure are not the same (250 kPa) in CHNS/O and CHN/O configurations with two furnaces, there is a negative peak between Nitrogen and Carbon peaks.

Solution: It is necessary to regulate both pressures to the same value to eliminate the negative peak and to have a good separation between Nitrogen and Carbon peaks.

Bad combustion of sample due to:

high weight of sample, required oxygen not sufficient, too many ashes catalyst exhausted.

Trouble:

Tailing on Nitrogen peak
Bad separation between Nitrogen and Carbon peaks
Split or tailing on C peak

Solution: Decrease the weight of sample, increase the oxygen quantity, clean the ashes, change the catalyst.

Influence of exhausted Copper

The function of the Copper is to reduce:

- To reduce Nitrogen Oxide to molecular Nitrogen,
- To reduce Sulphur Trioxide in Sulphur Dioxide
- To retain the excess of Oxygen used for the combustion.

During these redox reactions the Copper is converted in Copper Oxide.

Troubles: Bad separation between Nitrogen and Carbon Increasing Nitrogen blank value. Increasing in the Nitrogen data.

Solution: Replace the reactor.

Split on Hydrogen peak: Presence of fluorine containing compounds

The analysis of Fluorine-containing compounds shows many difficulties due to the high reactivity

Troubles:

Tailing or splitting on Hydrogen peak Ghost peaks on Carbon peak

Solution: For CHN configuration:

-fill a reactor with the specific absorber Fluorine mix

For CHNS configuration:

- to reduce the problem, weigh 0.4-0.6 mg of sample in a Silver capsule.

Analytical conditions

T tube: 950°C

T oven: 75°C

Helium Carrier Flow: 140 ml/min Helium Reference Flow: 100 ml/min

Oxygen Flow: 250 ml/min

Oxygen Injection Time: 5 sec

Sample Delay Time: 12 sec

Total Run time: less than 8 min

Standard: Acetanilide 0.8-1.2 mg

Calibration method: K factor Sample weight: 0.8-1.2 mg CHN reactor for Fluorine containing compounds

1 cm Quartz wool 3 cm Fluorine adsorber mix 3 cm Chromium Oxide

10 cm Copper

5 cm Silvered Cobaltous-Cobaltic Oxide 2 cm Quartz wool