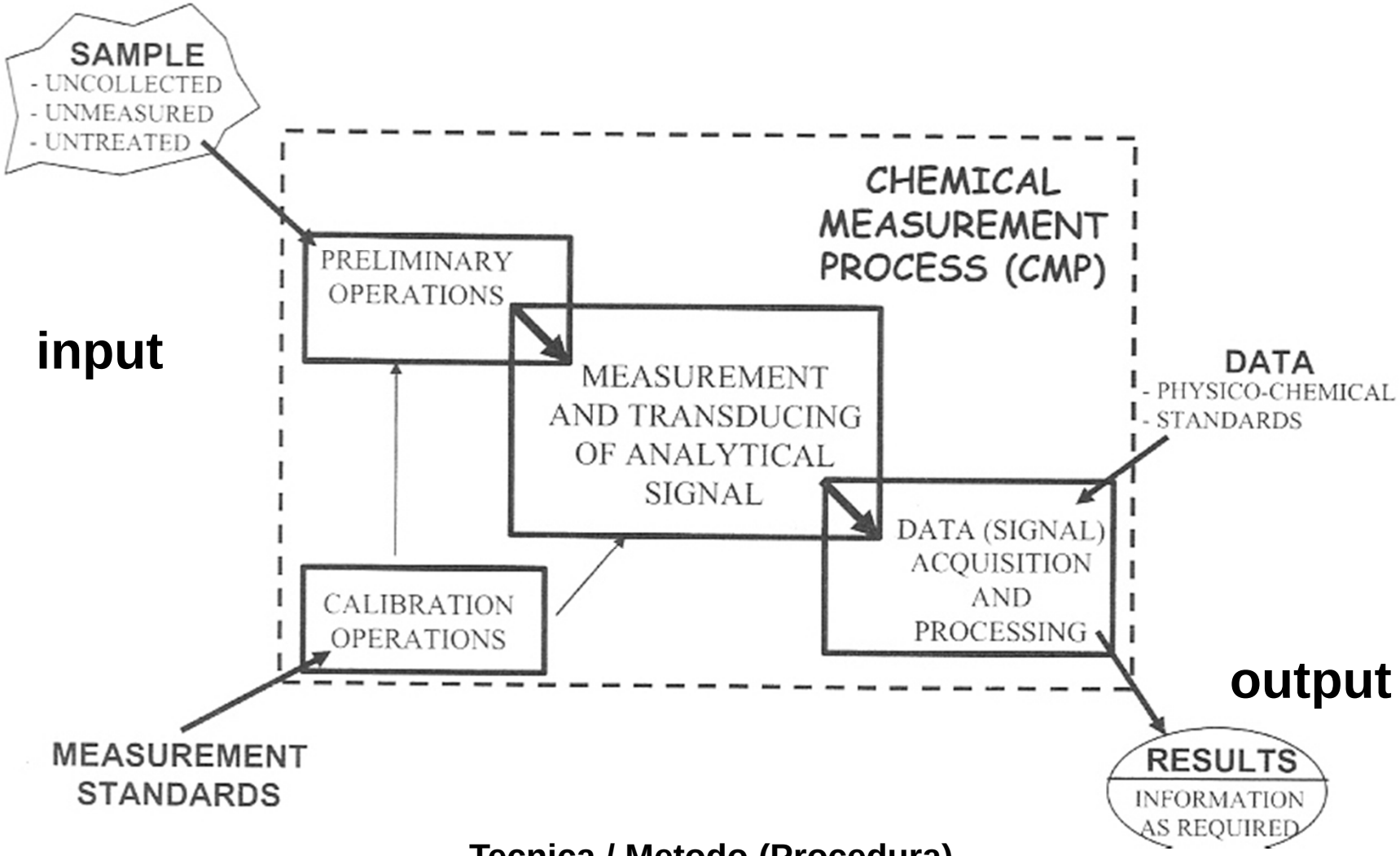
CHIMICA ANALITICA II
CON LABORATORIO

(AA 2016-17)

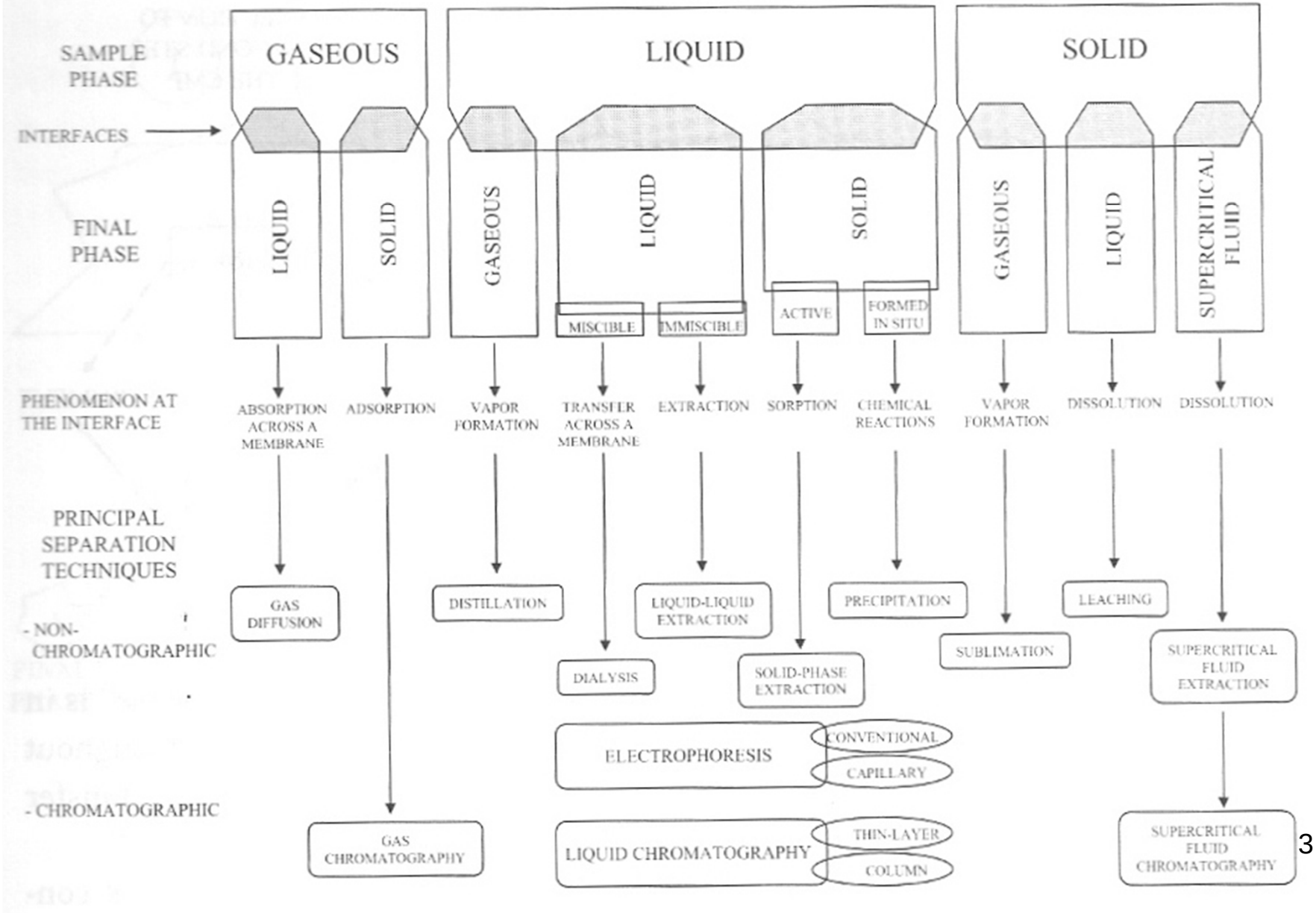
8 C.F.U. - Laurea triennale in Chimica

PASSI PRINCIPALI DI UN *Processo di Misura Chimico CMP* DAL CAMPIONE AI RISULTATI



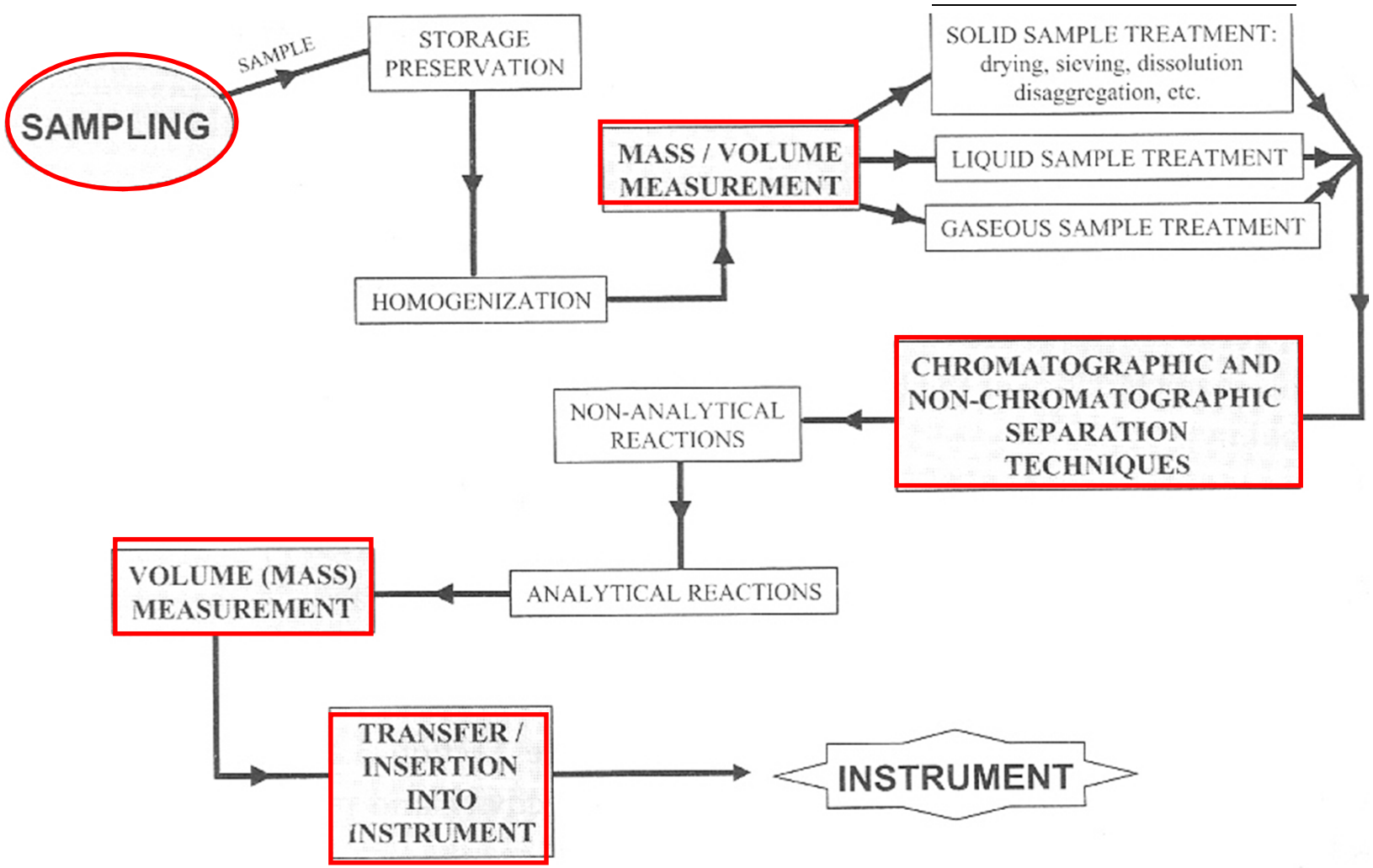
Tecniche di separazione

2) La preparazione del campione



2) La preparazione del campione

POSSIBILI PASSAGGI INTERMEDI DI UN *CMP*

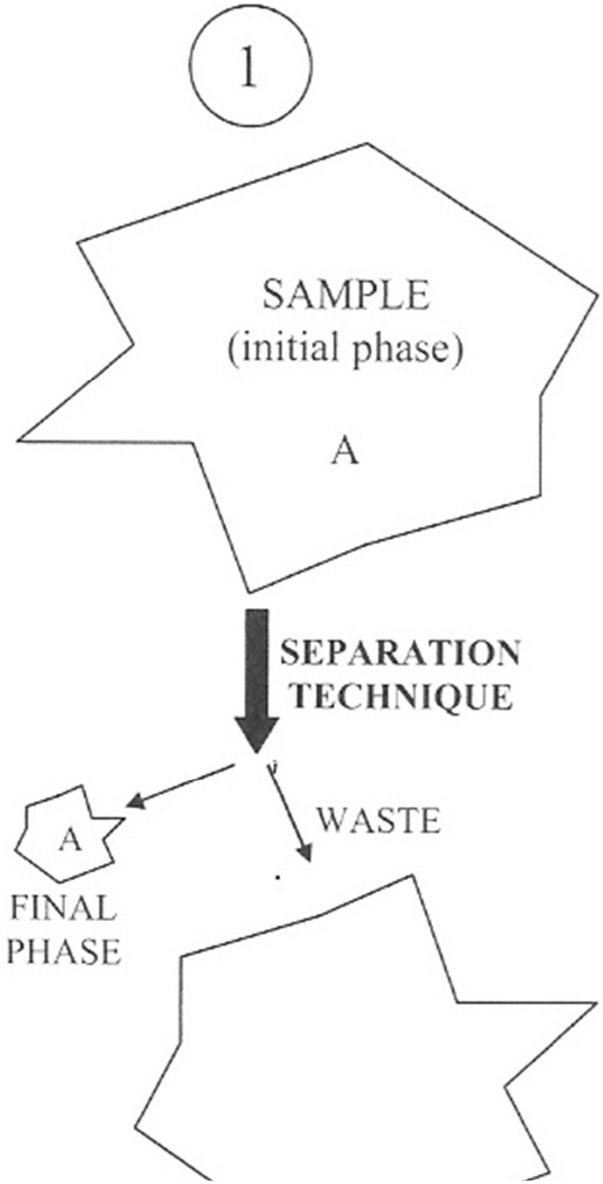


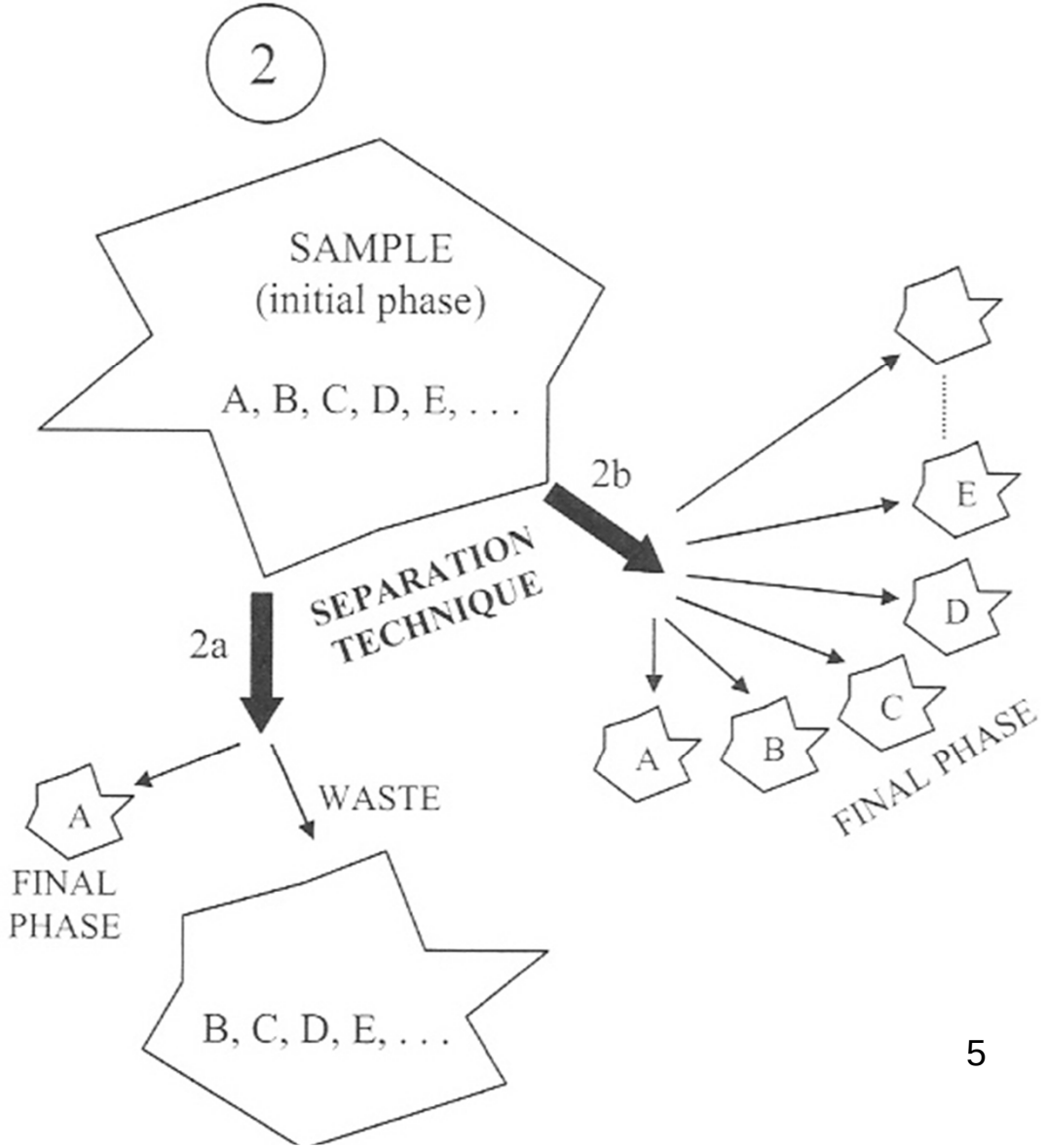
Coppia campione/analita; 70-90% del tempo in “step 1”

2) La preparazione del campione

Preconcetrazione

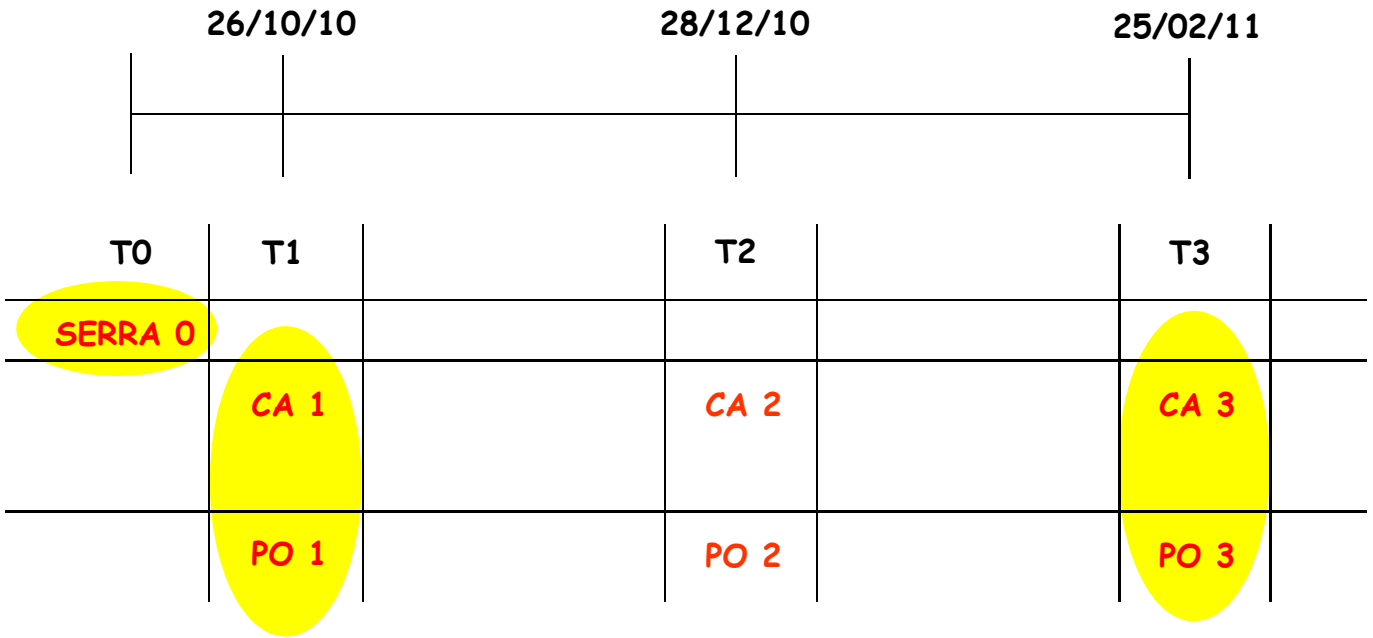
Clean-up





- Esempio: Valutazione quantitativa di IPA in materiale fogliare (Progetto UniUd - MiPAAF)

CAMPIONAMENTO PIANTE



A	B	D	E	G	H
Viburnum lucidum	Photinia x fraserii	Laurus nobilis	Ligustrum japonicum	Ilex aequifolium	Elaeagnus x ebbingei

5.2 Estrazione mediante sonicazione e purificazione dell'estratto

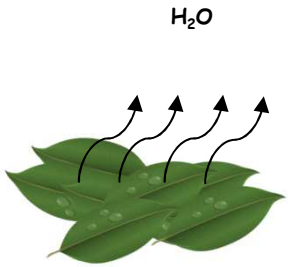
Il metodo di estrazione è stato adattato ai campioni forniti, partendo dai metodi di estrazione riportati da Orecchio et al. (Atmospheric Environment 41, 2007, 8669-8680) e De Nicola et al. (Atmospheric Environment 45, 2011, 1428-1433). Si è proceduto come segue.

- 1 g di campione secco è stato estratto per sonicazione in diclorometano (1 x 50 mL x 3 minuti, 2 x 30 mL x 3 minuti), dopo ogni sonicazione le foglie sono state recuperate tramite filtrazione sottovuoto e l'estratto conservato. Alla fine della procedura gli estratti sono stati riuniti e il volume ridotto a circa 1 mL tramite evaporazione a pressione ridotta.
- L'estratto ottenuto è stato purificato su colonna di Florisil (2g) eluendo con 15 mL di diclorometano e l'eluato concentrato a circa 0.5 mL tramite evaporazione a pressione ridotta e infine portato a secco tramite corrente di N2. Il residuo è stato infine ridisciolto in cicloesano (2 mL) e analizzato tramite GCMS.

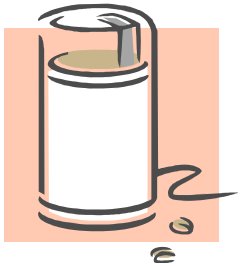
5.3 Aggiunta dello standard interno (pirene deuterato)

- La soluzione a concentrazione nota di pirene deuterato si aggiunge alla fine dell'iter preparativo e si utilizza come standard interno (IS) in modo da non dover lavorare con volumi esatti. Tipicamente si aggiungono all'estratto finale del campione 2000 ng assoluti di pirene-D.

ESTRAZIONE



Essiccazione in stufa a 40° C



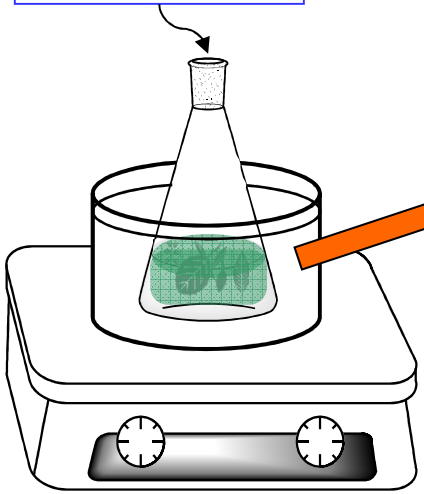
Macinazione

Conservazione
in congelatore,

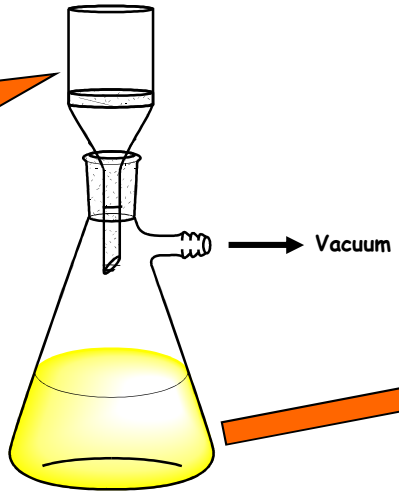
T amb, pesata

AGGIUNTA IPA-D!

1 g campione secco



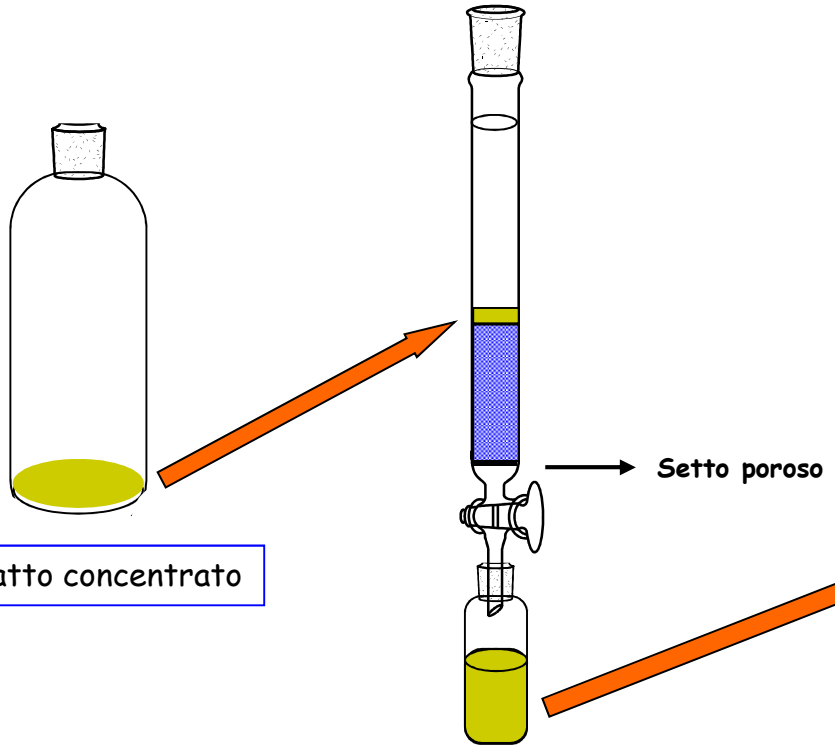
Sonicazione in DCM



Filtrazione sottovuoto

Concentrazione sottovuoto

PURIFICAZIONE



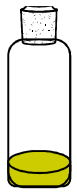
Estratto concentrato

Purificazione in colonna su:

Florisil®

SiO_2 (84.0 ±0.5%)
MgO (15.5 ±0.5%)
Na_2SO_4 (≤1.0%)

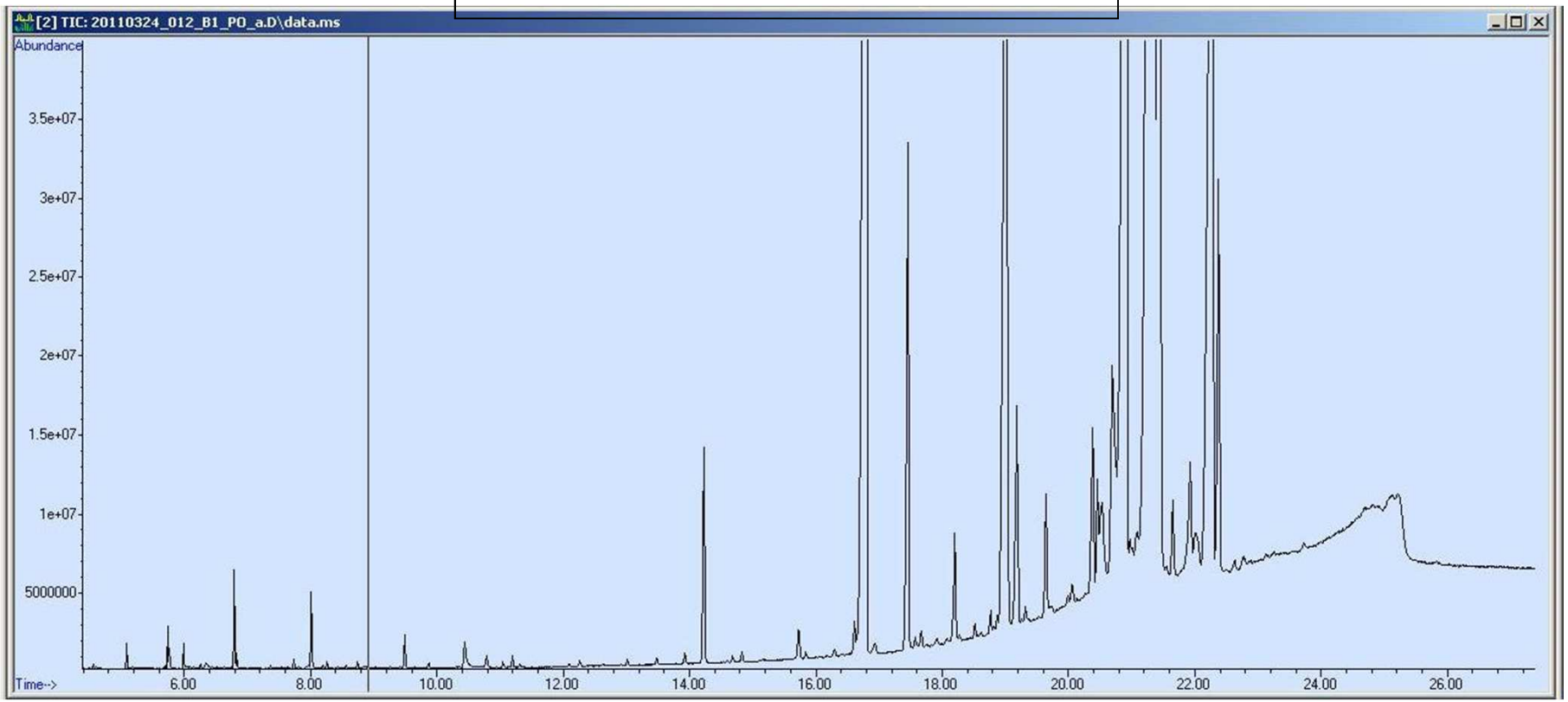
Concentrazione sottovuoto



AGGIUNTA Pirene-D!

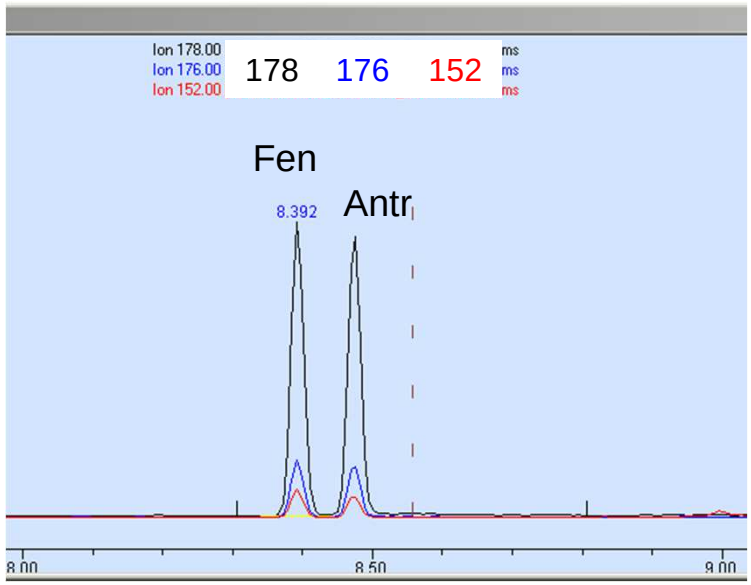
Trasferimento in vial per GC-MS

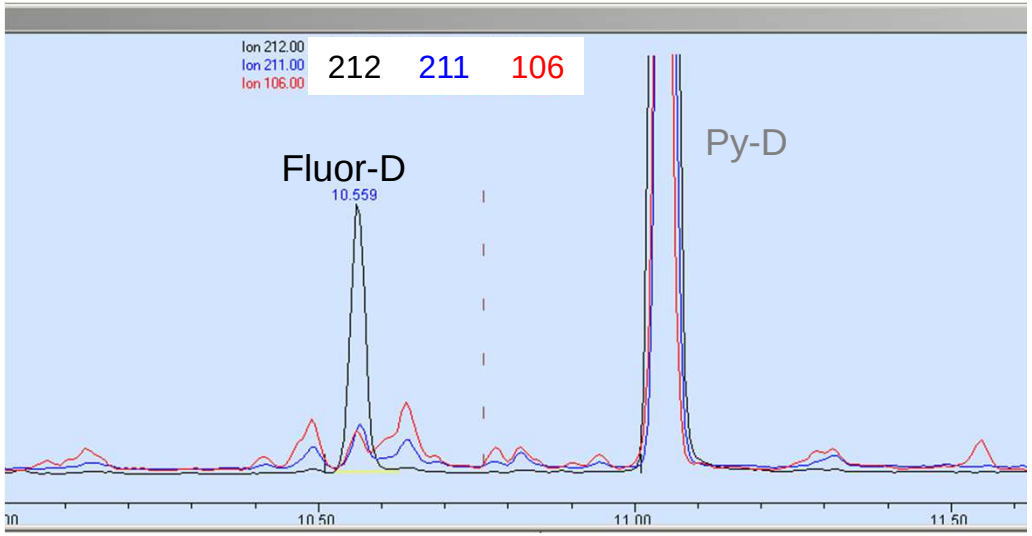
CROMATOGRAMMA

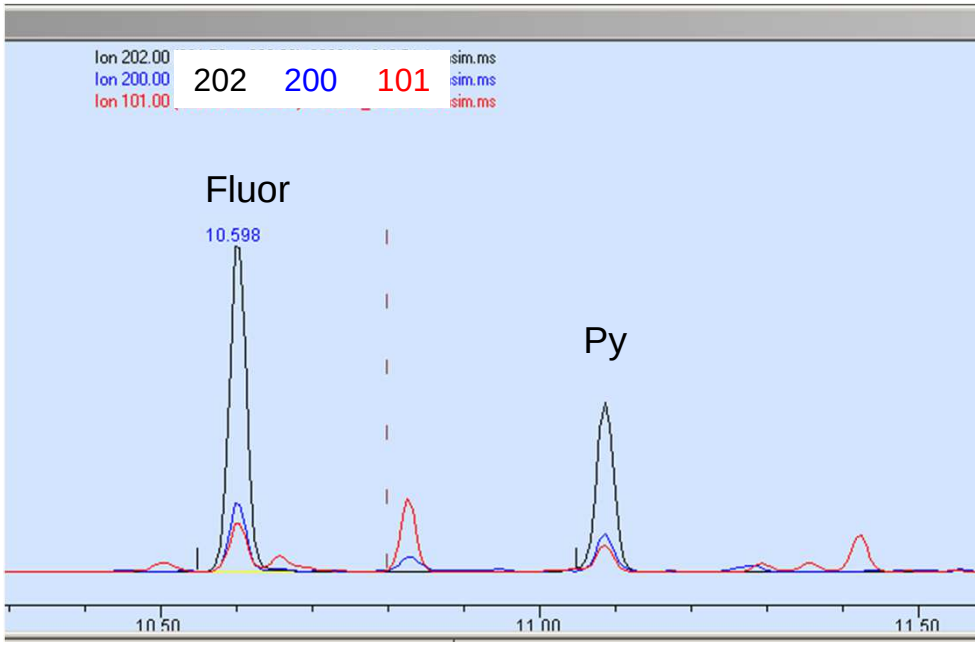


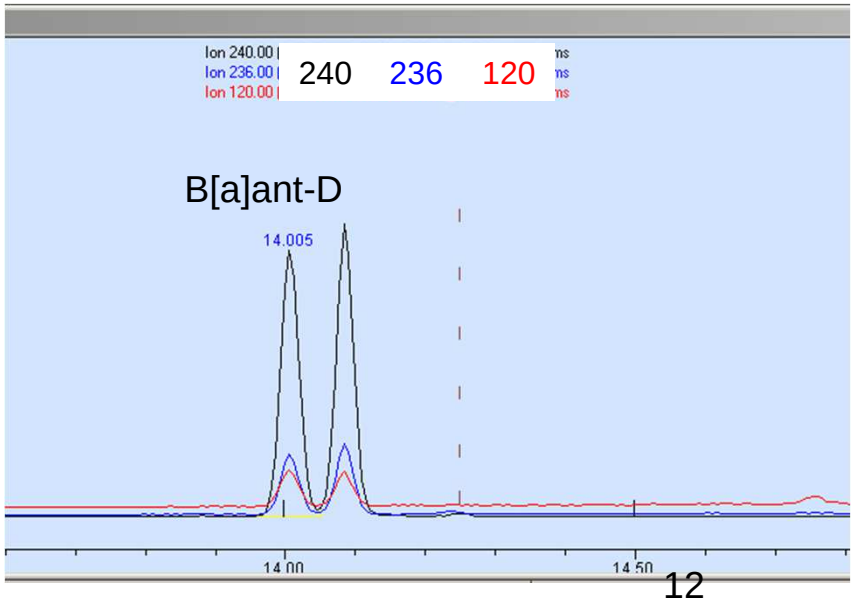
TIC: Total Ion Current

SIM: Single Ion Monitoring


Ion 178.00
Ion 176.00
Ion 152.00
178
176
152
Fen
Antr
8.392
8.00
8.50
9.00


Ion 212.00
Ion 211.00
Ion 106.00
212
211
106
Fluor-D
10.559
Py-D
10.50
11.00
11.50

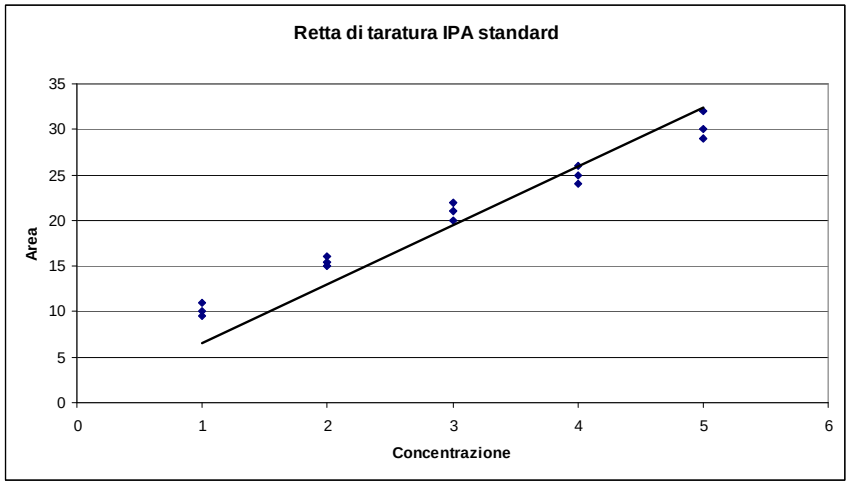

Ion 202.00
Ion 200.00
Ion 101.00
202
200
101
Fluor
10.598
Py
10.50
11.00
11.50


Ion 240.00
Ion 236.00
Ion 120.00
240
236
120
B[a]ant-D
14.005
14.00
14.50

ANALISI IN GC-MS

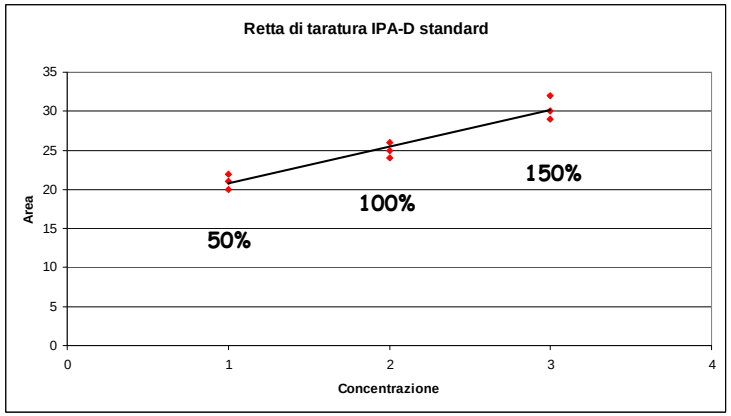
ANALISI QUANTITATIVA:

Retta di calibrazione utilizzando soluzione standard di IPA



CALCOLO DEI RECUPERI:

Retta di calibrazione utilizzando soluzione standard di IPA-D



PER NORMALIZZARE LE CONCENTRAZIONI:

Aggiunta di un'aliquota di Pirene-D (soluzione standard) PRIMA del trasferimento in vial per GC-MS13

Trasduzione

trasduzióne s. f. [der. di trasdurre]. –

- 1. a. Nel linguaggio tecn., trasmissione di energia da un punto a un altro di un sistema, soprattutto quando i livelli energetici siano bassi, ovvero si tratti di segnali informatici per misure, controlli, ecc. (in caso di elevate quantità di energia si preferisce parlare di conversione): dispositivo, linea di trasduzione.
- b. Con sign. più specifico, il termine indica ***processi di trasmissione che sono accompagnati da una modificazione della natura dell'energia trasmessa***: per es., trasmissione di energia meccanica convertita in energia elettrica o viceversa (t. elettromeccanica; in partic., t. elettroacustica), di energia luminosa convertita in energia elettrica o viceversa (t. fotoelettrica o elettroottica), e così via (v. anche ***trasduttore***).

trasduttóre s. m. [der. di trasdurre, per traduz. dell'ingl. transductor].

– Nel linguaggio tecn., denominazione generica di ***ogni dispositivo atto a ricevere segnali di determinata natura da un mezzo di trasmissione trasformandoli in altri segnali generalmente di diversa natura, che possono essere trasmessi attraverso un altro mezzo di trasmissione***; a seconda della natura dei segnali d'ingresso e di quelli d'uscita, si hanno: t. meccanoelettrici (e in partic. acustoelettrici o fonoelettrici, piezoelettrici, ecc.), elettromeccanici (e in partic. elettroacustici), fotoelettrici, elettroottici, ecc.; quelli i cui segnali d'uscita sono di natura elettrica sono brevemente detti t. elettrici

SEVEN STAGES OF AN ANALYTICAL METHOD

3) La misura e la trasduzione del segnale analitico

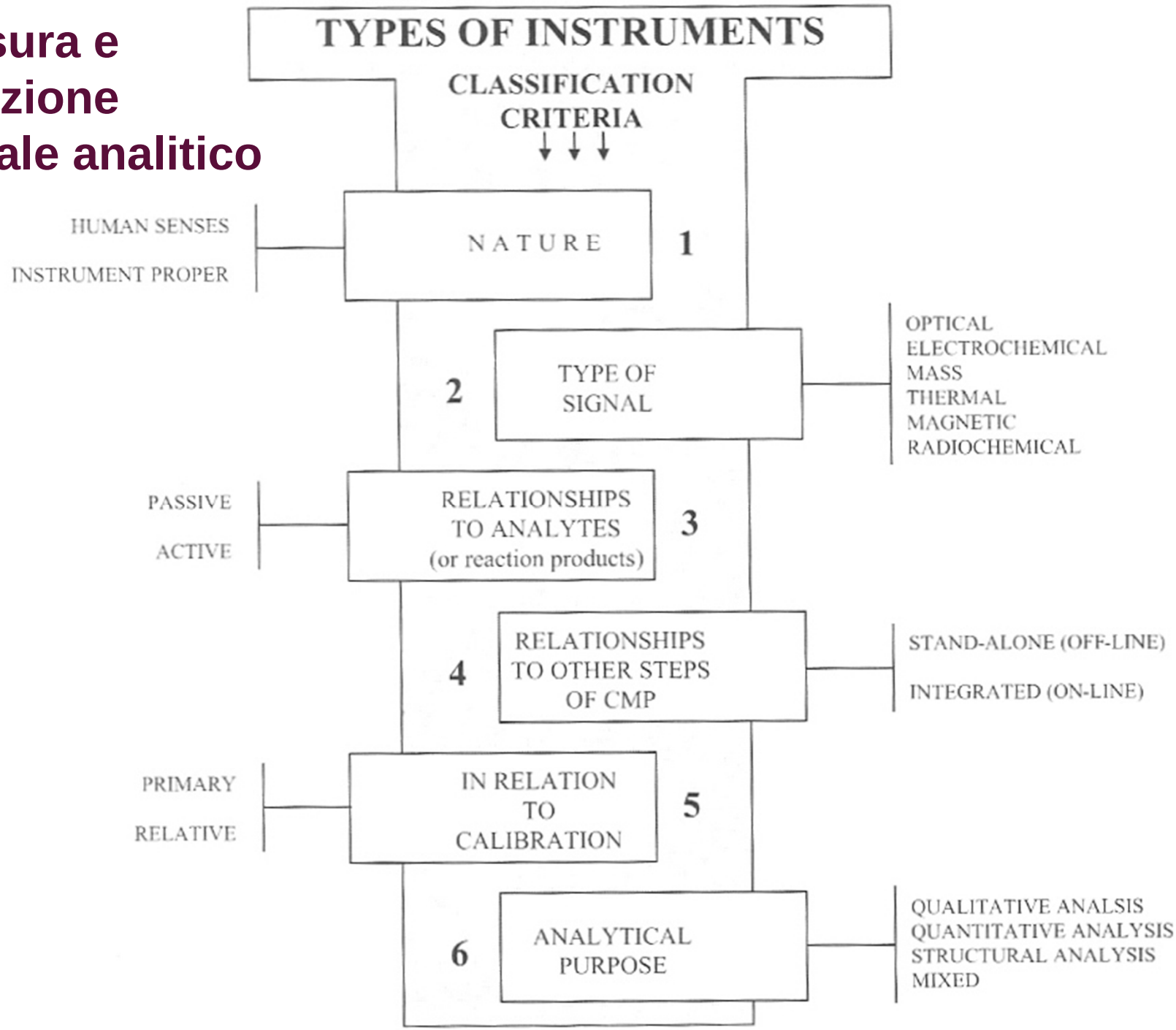
1. **Conception** of analytical method (***birth***).
2. Successful **demonstration** that the analytical method works.
3. **Establishment** of the analytical method's capabilities.
4. Widespread **acceptance** of the analytical method.
5. Continued **development** of the analytical method leads to significant improvements.
6. **New cycle** through steps 3–5.
7. Analytical method can no longer compete with newer analytical methods (***death***).

Steps 1–3 and 5 are the province of *analytical chemistry*; step 4 is the realm of *chemical analysis.*

modified from Fassel, V. A. Fresenius' Z. Anal. Chem. 1986, 324, 511–518 and Hieftje, G. M. J. Chem. Educ. 2000, 77, 577–583.

https://dl.dropboxusercontent.com/u/9630480/Site/eTextProject/pdfFiles/AnalChem2.0.pdf

3) La misura e la trasduzione del segnale analitico



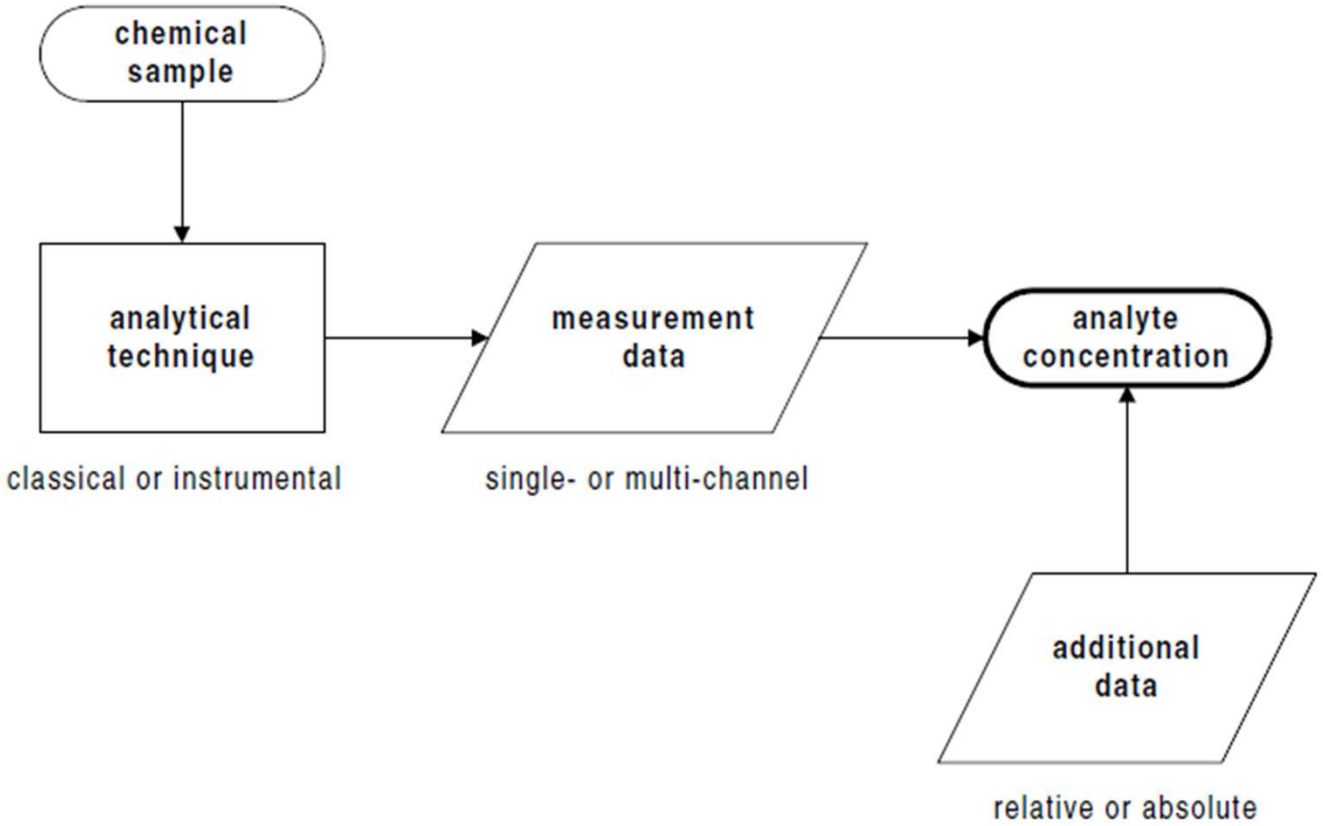


Figure 1: Schematic showing measurement steps involved in quantitative chemical analysis of a sample. There are three ways of classifying the process, based on the technique (classical *vs* instrumental), the measurement data (single-channel *vs* multi-channel), or on whether additional data is needed to estimate the analyte concentration (relative *vs* absolute).

https://facultystaff.richmond.edu/~rdominey/301/local/Intro_Instrum_Analysis.pdf

There are a very large number of techniques used in chemical analysis. It can be very useful to classify the measurement process according to a variety of criteria:

- by the type of analytical technique – ***classical*** or ***instrumental*** techniques;
- by the nature of the measurement data generated – ***single-channel*** or ***multi-channel*** techniques; and
- by the quantitation method (by which the analyte concentration is calculated) – ***relative*** or ***absolute*** techniques.

Classical *vs* Instrumental Techniques

In *classical* analysis, the signal depends on the chemical properties of the sample: a reagent reacts completely with the analyte, and the relationship between the measured signal and the analyte concentration is determined by chemical stoichioimetry. In ***instrumental*** analysis, some physical property of the sample is measured, such as the electrical potential difference between two electrodes immersed in a solution of the sample, or the ability of the sample to absorb light.

Classical methods are most useful for accurate and precise measurements of analyte concentrations at the 0.1% level or higher. On the other hand, some specialized instrumental techniques are capable of detecting individual atoms or molecules in a sample! Analysis at the ppm (μg/mL) and even ppb (ng/mL) level is routine.

The advantages of instrumental methods over classical methods include:

1. The ability to perform *trace analysis*, as we have mentioned.
2. Generally, large numbers of samples may be analyzed very quickly.
3. Many instrumental methods can be automated.
4. Most instrumental methods are multi-channel techniques (we will discuss these shortly).
5. Less skill and training is usually required to perform instrumental analysis than classical analysis.

CLASSICAL ANALYSIS

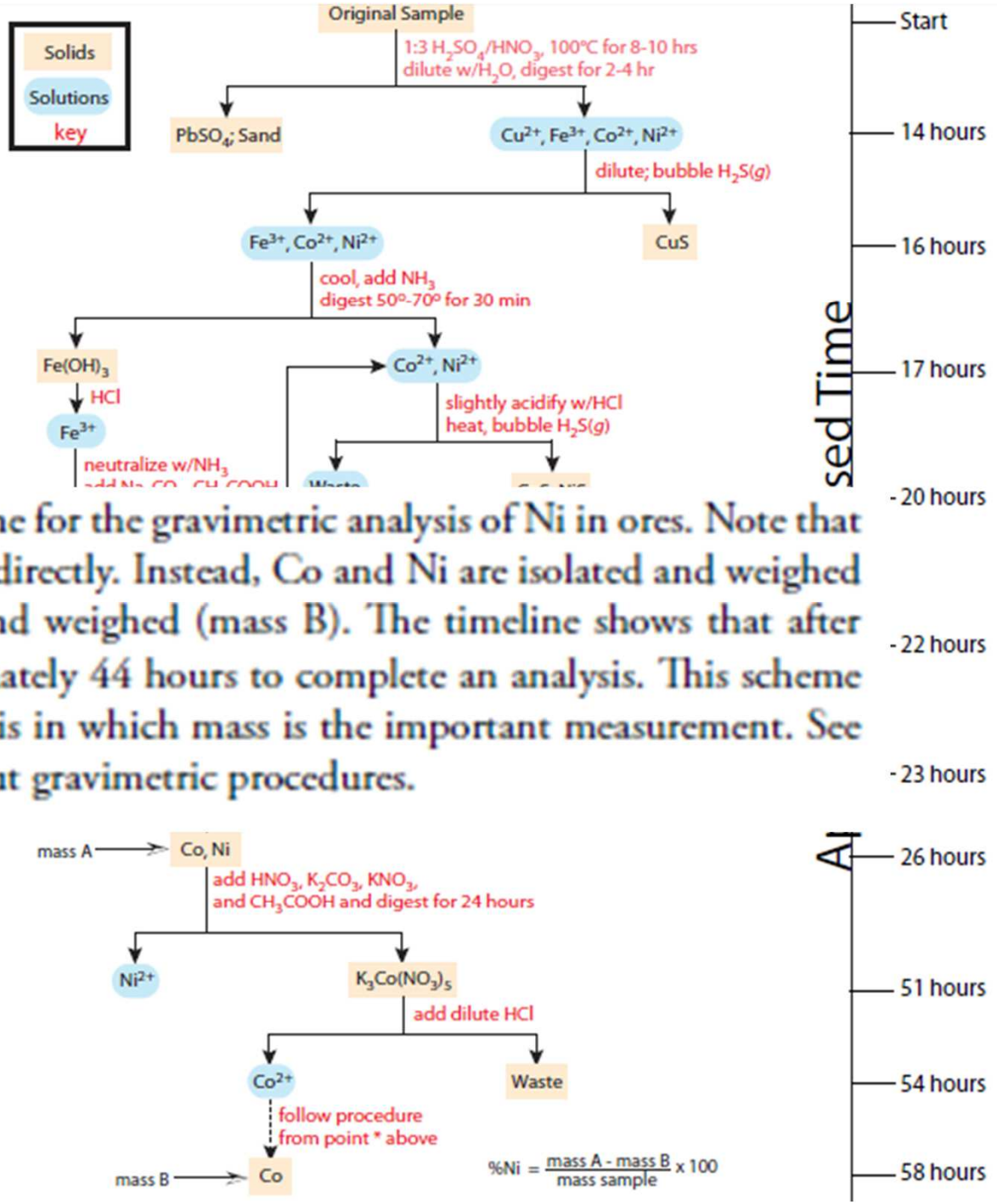


Figure 1.1 Fresenius' analytical scheme for the gravimetric analysis of Ni in ores. Note that the mass of nickel is not determined directly. Instead, Co and Ni are isolated and weighed (mass A), and then Co is isolated and weighed (mass B). The timeline shows that after digesting a sample, it takes approximately 44 hours to complete an analysis. This scheme is an example of a gravimetric analysis in which mass is the important measurement. See Chapter 8 for more information about gravimetric procedures.

1905

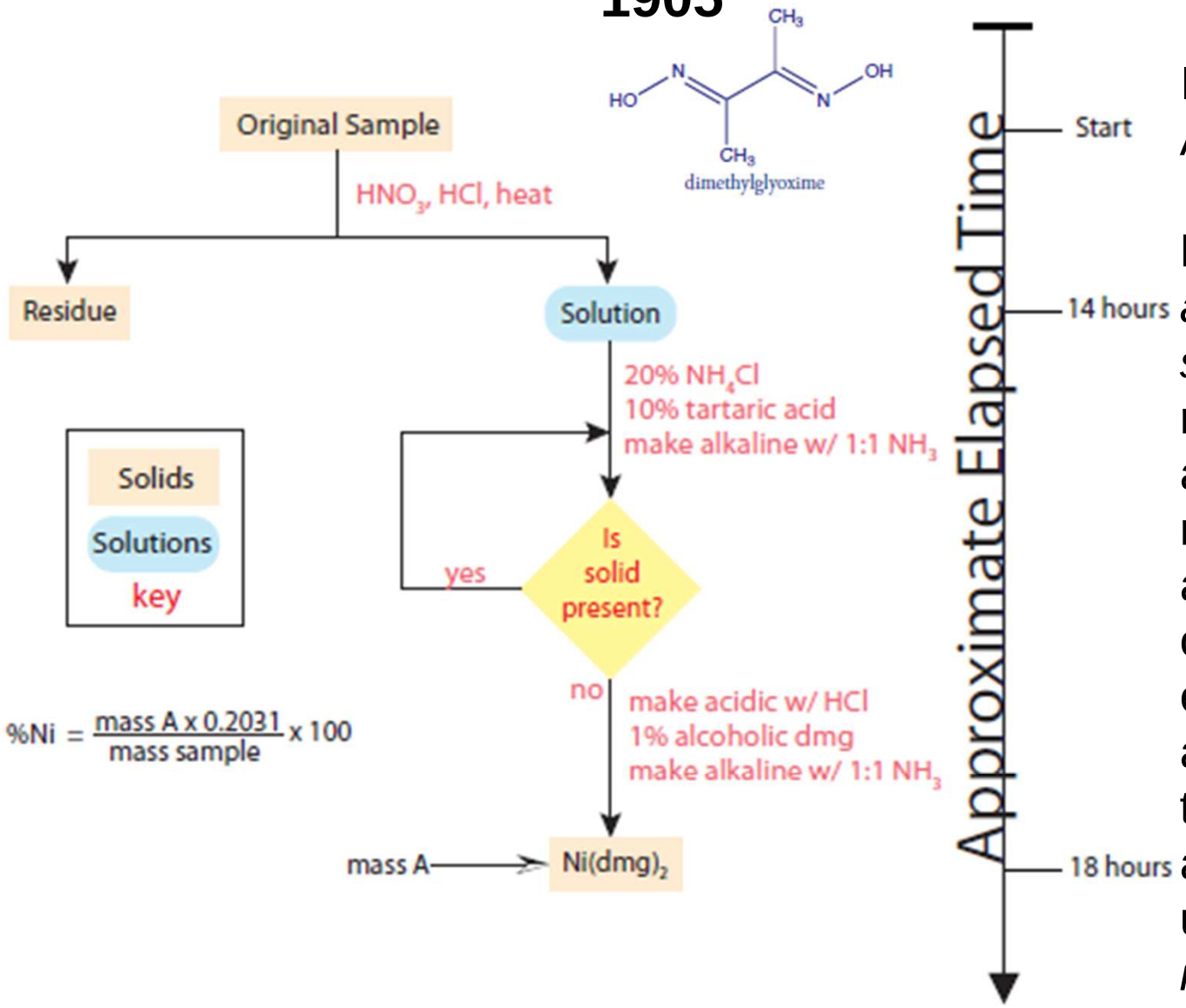


Figure 1.2 Gravimetric analysis for Ni in ores by precipitating $Ni(dmg)_2$. The timeline shows that it takes approximately four hours to complete an analysis after digesting the sample, which is 10x shorter than for the method in Figure 1.1. The factor of 0.2301 in the equation for %Ni accounts for the difference in the formula weights for Ni and $Ni(dmg)_2$; see Chapter 8 for further details.

INSTRUMENTAL ANALYSIS

By the **1970s**, flame *atomic absorption spectrometry* replaced gravimetry as the standard method for analyzing nickel in ores, resulting in an even more rapid analysis. **Today**, the standard analytical method utilizes an *inductively coupled plasma optical emission spectrometer*.

Instrumental analysis can be further classified according to the principles by which the measurement signal is generated. A few of the methods are listed below. [The underlined methods are to be used in the round-robin experiments.]

1. ***Electrochemical*** methods of analysis, in which the analyte participates in a redox reaction or other process. In potentiometric analysis, the analyte is part of a galvanic cell, which generates a voltage due to a drive to thermodynamic equilibrium. The magnitude of the voltage generated by the galvanic cell depends on the concentration of analyte in the sample solution. In voltammetric analysis, the analyte is part of an electrolytic cell. Current flows when voltage is applied to the cell due to the participation of the analyte in a redox reaction; the conditions of the electrolytic cell are such that the magnitude of the current is directly proportional to the concentration of analyte in the sample solution.
2. ***Spectrochemical*** methods of analysis, in which the analyte interacts with electromagnetic radiation. Most of the methods in this category are based on the measurement of the amount of light absorbed by a sample; such *absorption-based* techniques include atomic absorption, molecular absorption, and nmr methods. The rest of the methods are generally based on the measurement of light emitted or scattered by a sample; these *emission-based* techniques include atomic emission, molecular fluorescence, and Raman scatter methods.
3. The technique of ***mass spectroscopy*** is a powerful method for analysis in which the analyte is ionized and subsequently detected. Although in common usage, the term "spectroscopy" is not really appropriate to describe this method, since electromagnetic radiation is not usually involved in mass spectroscopy. Perhaps the most important use of mass spectrometers in quantitative analysis is as a gas or liquid chromatographic detector. A more recent innovation is the use of an inductively coupled plasma (ICP) as an ion source for a mass spectrometer; this combination (ICP-MS) is a powerful tool for elemental analysis.

Single-Channel *vs* Multi-Channel Techniques

So now we have classified analytical methods according to the method by which they generate the measurement data. Another useful distinction between analytical techniques is based on the information content of the data generated by the analysis:

- ***single-channel*** techniques will generate but a single number for each analysis of the sample. Examples include gravimetric and potentiometric analysis. In the former, the signal is a single mass measurement (e.g., mass of the precipitate) and in the latter method the signal is a single voltage value.
- ***multi-channel*** techniques will generate a series of numbers for a single analysis. Multi-channel techniques are characterized by the ability to obtain measurements while changing some independently controllable parameter. For example, in a molecular absorption method, an absorption *spectrum* may be generated, in which the absorbance of a sample is monitored as a function of the wavelength of the light transmitted through the sample. Measurement of the sample thus produces a series of absorbance values.

Any multi-channel technique can thus produce a plot of some type when analyzing a single sample, where the signal is observed as a function of some other variable: absorbance as a function of wavelength (in molecular absorbance spectroscopy), electrode potential as a function of added titrant volume (potentiometric titrimetry), diffusion current as a function of applied potential (voltammetry), etc. Multi-channel methods provide a lot more data – and information – than single-channel techniques.

Multi-channel methods have two important advantages over their single-channel counterparts:

1. They provide the ability to perform *multicomponent analysis*. In other words, the concentrations of more than one analyte in a single sample may be determined.

2. Multi-channel methods can detect, and sometimes correct for, the presence of a number of types of interferences in the sample. If uncorrected, the presence of the interference will result in biased estimates of analyte concentration.

Multi-channel measurements simply give more information than a single-channel signal. For example, imagine that measurement of one of the calibration standards gives the data pictured in fig 2(a):

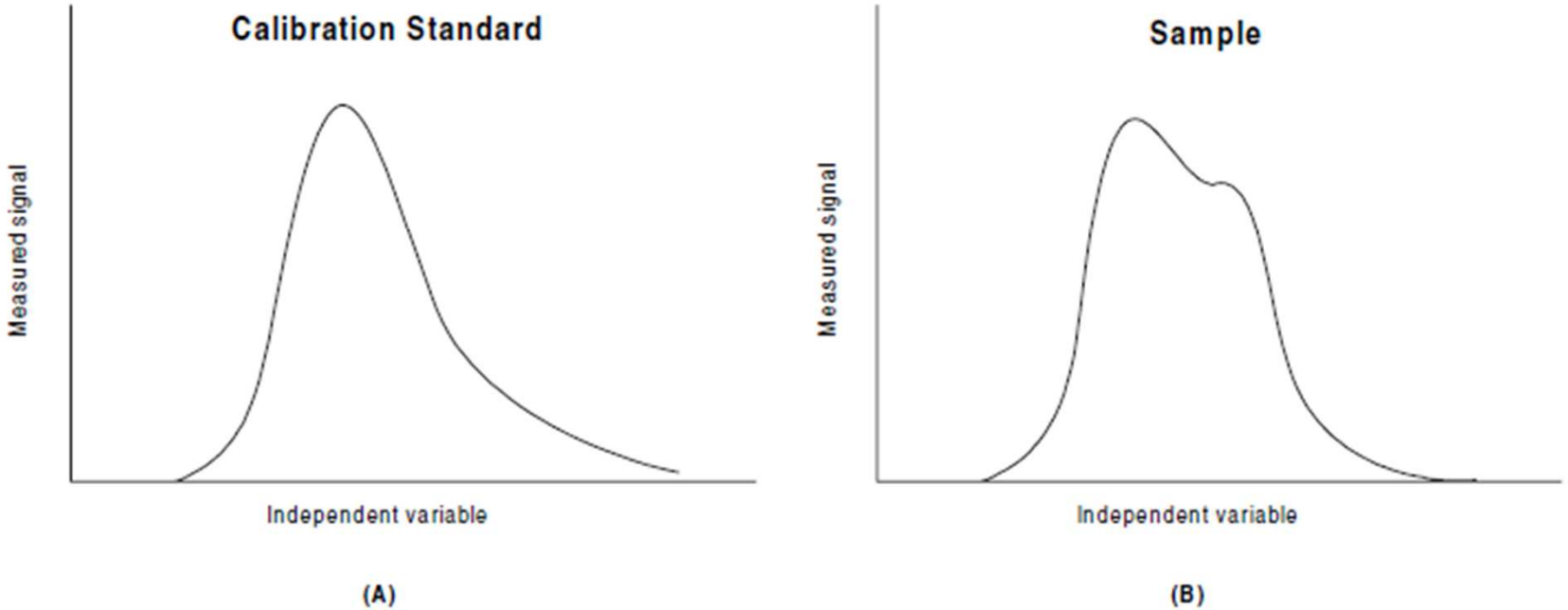


Figure 2: illustration of how multi-channel data allow for the detection of interferences. Comparison of the multi-channel signal of the (a) the calibration standard, and (b) the sample reveals that there is interference in the latter. A likely explanation is that another component of the sample (absent from the calibration standard) also gives a measurable response. The left side of the peak appears relatively unaffected by the presence of the interferent; it may be possible to obtain an unbiased estimate of analyte concentration by using one of these channels for quantitation.

Plots of the measurements of the other calibration standards (assuming they are not contaminated) should give the same general shape, although the magnitude of the signal will of course depend on the analyte concentration.

Now imagine that you obtain multi-channel measurements of a sample, recording the following data shown in fig 2(b). It is immediately obvious that the shape has changed due to some interference. A likely explanation is that some component of the sample matrix is also contributing to the measured signal, so that the result is the sum of the two (or perhaps more than two) sample components. Another possibility is that the sample matrix alters the response of the analyte, giving rise to an altered peak shape.

Relative *vs* Absolute Techniques

Another way of classifying analytical techniques is according to the method by which the analyte concentration is calculated from the data:

- in ***absolute*** analytical techniques, the analyte concentration can be calculated directly from measurement of the sample. No additional measurements are required (other than a measurement of sample mass or volume).

- in ***relative*** analytical techniques, the measurement of the sample must be compared to measurements of additional samples that are prepared with the use of analyte *standards* (e.g., solutions of known analyte concentration).

The following figure illustrates the difference between the two types of methods.

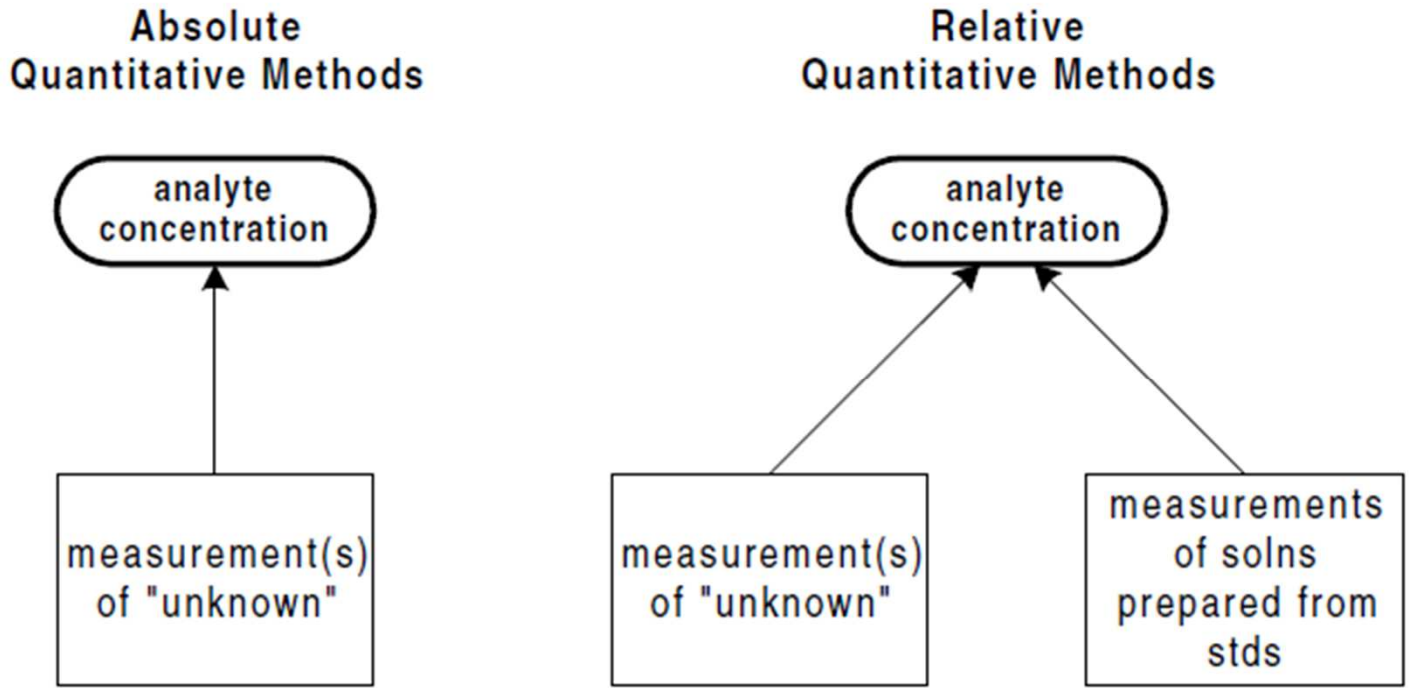
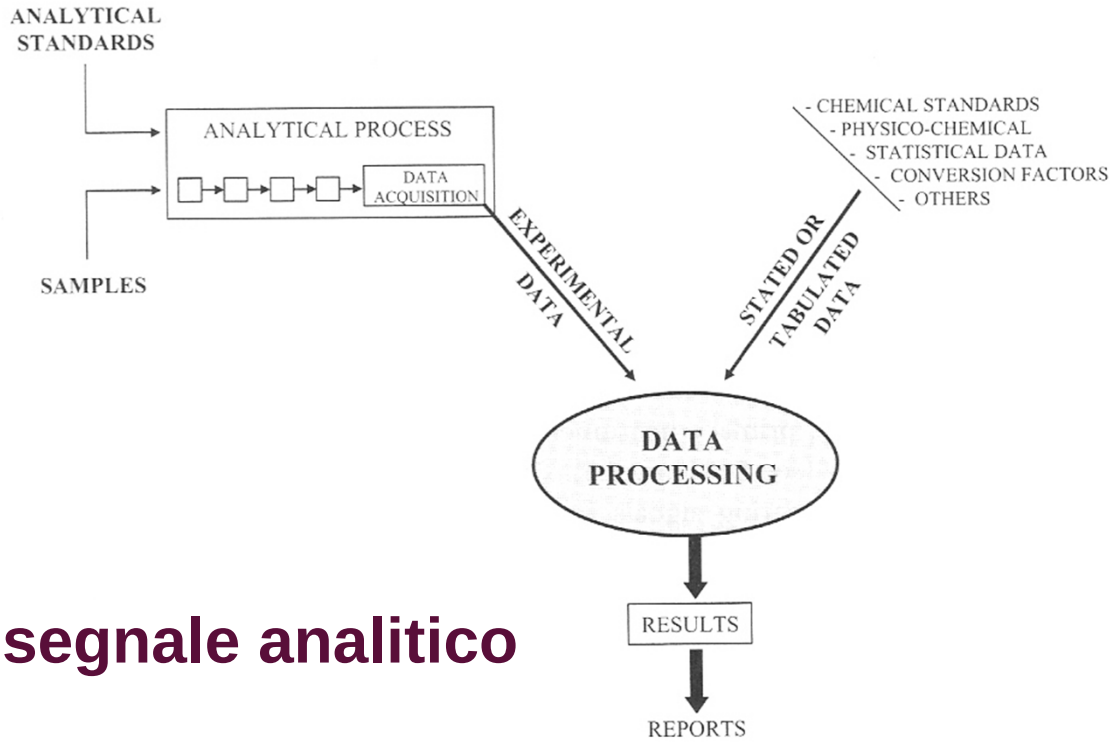


Table 1: Characterization of analytical techniques discussed in this course. For each technique, the table states the property being measured (second column); for multi-channel techniques, the independent parameter is also given. So, for example, we can see that in potentiostatic coulometry (6th row), current is measured as a function of time. The last column states how the measured quantity is determined by the analyte concentration in the sample.

Technique	Quantity Measured	Single- or multi-channel? (*independent parameter*)	Theoretical Principle
Classical Techniques – all absolute methods[a]			
gravimetry	mass	single-channel	complete/selective rxn of analyte; composition of weighing form is known
electrogravimetry	mass	single-channel	
titrimetry (chemical indicator)	endpoint volume/mass	single-channel	complete/selective rxn of analyte; known stoichiometry of titration reaction
titrimetry (instrum endpt detection)	instrument signal	multi-channel (*volume/mass of titrant solution*)	
amperostatic coulometry	time	single-channel	complete/selective rxn of analyte; Faraday's Law, and the known stoichiometry of titration reaction
potentiostatic coulometry	current	multi-channel (*time*)	
Instrumental Techniques – all relative methods[b]			
potentiometry	potential	single-channel	thermodynamic drive to equilibrium (Nernst)
voltammetry	current	multi-channel (*working electrode potential*)	analyte diffusion controls signal (Fick's Law)
atomic absorption	attenuation of light intensity	single-channel[c]	Beer's Law
molecular absorption	attenuation of light intensity	multi-channel (*wavelength*)	
atomic emission	emitted light intensity	multi-channel (*wavelength*)	signal is proportional to excited-state concentration
molecular fluorescence	fluorescence light intensity	multi-channel[d] (*excitation wavelength* and *emission wavelength*)	

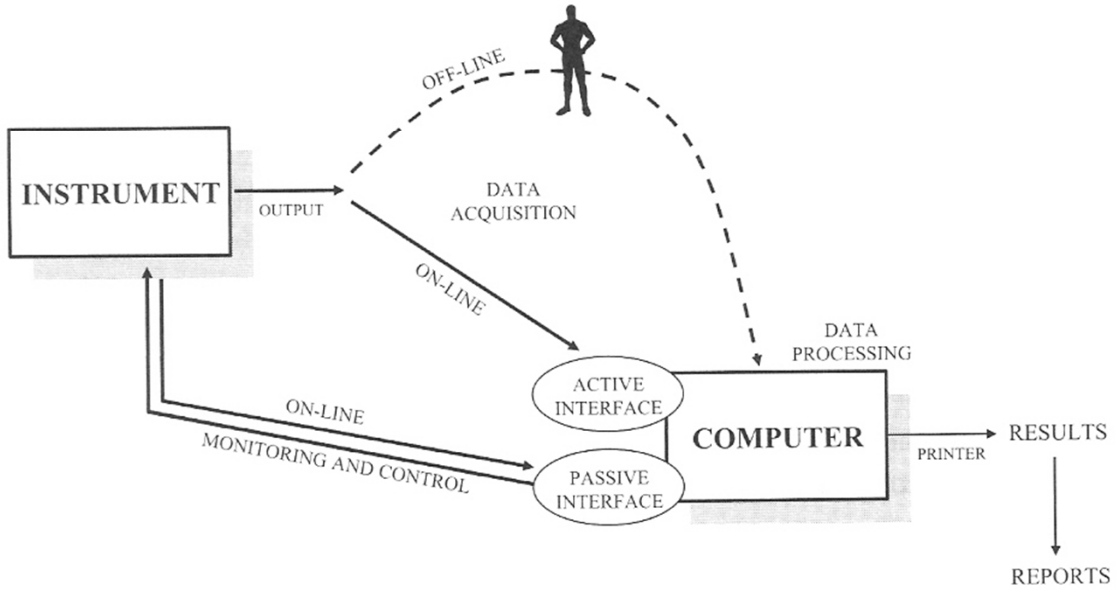
[a]strictly speaking, titrimetry is only an absolute method if the titrant solution is prepared from a primary standard

Figure 4.18. The two main sources of data to be processed in order to produce analytical results as required in the third step of a CMP



3) La misura e la trasduzione del segnale analitico

Figure 4.19. Possible relationships between an instrument and a computer in the third step of the analytical process; for details, see text



Methods of Quantitation for Instrumental Analysis

Instrumental techniques are almost all *relative* in nature: the signal obtained from the analysis of the sample must be compared to other measurements in order to determine the analyte concentration in the sample. Since these other measurements naturally contain measurement error, relative quantitation increases the overall error in the estimate of analyte concentration – we shall refer to this source of error as ***calibration error***. Calibration error can contain both random and systematic components. One of the advantages of classical methods over instrumental methods is the absence of calibration error, since classical methods are *absolute* in nature.

Classical methods are absolute because of the direct relationship between the quantity measured and the analyte concentration. Why isn't the same thing true of instrumental methods? There are a wide variety of instruments used for analysis, and they can generally be broken down into four components:

1. A *signal generator*, in which the analyte in the sample results in the production of some form of energy (such as light or heat);
2. A *transducer*, or "detector," that transforms the energy produced in the signal generator into an electrical signal (usually a voltage or a current);
3. Various *electronic components*, such as amplifiers and filters, that "clean up" the electrical signal; and
4. A *read-out device*, such as a chart recorder, an analog meter, an oscilloscope or a computer, that converts the electrical signal into a form that is usable by the analyst.

For example, let's consider the process involved in the method of *flame atomic emission*, in which the analyte solution is "sprayed" into a hot combustion flame.

- the analyte atoms will absorb thermal energy from the flame, and will release some of this energy in the form of light. The light is collected by and separated into its component wavelengths. This part of the instrument can be considered the "signal generator," because the amount of light at a certain wavelength will be proportional to the concentration of analyte atoms in the flame.
- light of the proper color (i.e., light emitted by the analyte atoms) is directed to strike a photon detector, which produces a current.
- this photon-induced current is amplified and converted into a voltage
- the voltage is then used to drive the pen of a strip-chart recorder.

This description illustrates that there is no simple relationship between the data produced by the read-out device of an analytical instrument and the concentration of the analyte in the chemical sample. This is in contrast to the case in classical analysis, where the relationship between measured values such as mass or volume and the analyte concentration is fairly direct, and can be calculated from the stoichiometry of the chemical reaction.

To see why the relationship between signal and analyte concentration in instrumental analysis is so complicated, let's go back to our example of flame atomic emission, where:

- the distance moved by the pen in the chart recorder is proportional to the voltage output of the electronics in the instrument;
- the voltage output is proportional to the current produced by the light detector;
- the current produced by the light detector is proportional to the light intensity striking the detector;
- the light intensity striking the detector is proportional to the intensity of light emitted by the analyte atoms in the flame;
- the emission intensity from the flame is proportional to the number of analyte atoms in the flame; and finally
- the number of analyte atoms in the flame is proportional to the concentration of analyte in the sample solution.

Although the end result of all this hand waving is that the data produced by the instrument is proportional to the analyte concentration in the sample, it is not a relationship that is readily amenable to theoretical treatment. Instead, we must estimate the analyte concentration in the sample by using solutions of known analyte concentration.

The two most common methods of calibration in instrumental analysis are (i) the use of ***calibration curves***, and (ii) the ***method of standard additions***. In addition, ***internal standards*** may be used in combination with either of these methods. We will now describe how these methods may be used in quantitative chemical analysis.

Trends scientifici e tecnici

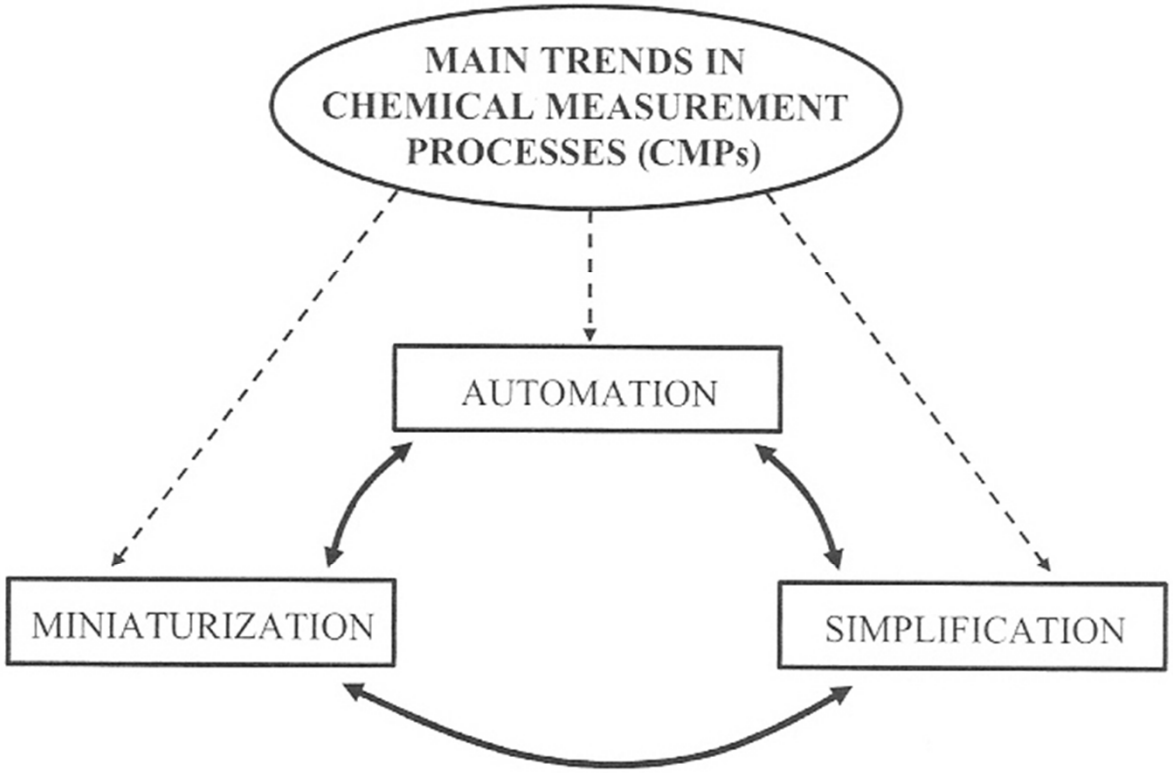


Figure 4.20. Principal scientific and technical trends in the design and application of new chemical measurement processes

… ma anche…

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