LEARNING TOXICOLOGY THROUGH OPEN EDUCATIONAL RESOURCES

ENVIRONMENTAL SAMPLING AND ANALYTICAL MEASUREMENTS

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1. INTRODUCTION

Following the environmental monitoring flow, this unit will outline how to implement the execution stage (Figure 1.):

- principles of sampling and sample preparation for environmental analysis (U 2.1);
- principles of analytical methods applicable for pollutants determination in environment and quality requirements for environmental analysis (U 2.2).

Figure 1. Environmental monitoring flow, execution and evaluation stages.

At the end of the Unit 2, students will be able to:

- describe the principles of the methods available for environmental sampling and sample preparation;
- select the sampling and sample preparation methods that are appropriate for a specific environmental sample'
- describe the principles of the analytical methods available for environmental analysis;
- identify and select the analytical methods appropriate for pollutants measurements from environmental samples;
- describe introductory concepts and applications of metrology in chemistry for environmental analysis.

There are several areas for which sampling and environmental analyses are required:

1. **routine monitoring –** for example to measure concentration of pollutants in the environment and to identify short- and long-term trends;
2. **emergency response** – for example to detect accidental release of pollutants in the environment and to evaluate risk and toxicity of humans and biota;
3. **regulatory enforcement and regulatory compliance** – for example to measure how much pollutants are released into environment (gaseous

emissions, wastewater discharge) in order to comply with regulatory requirements;

4. **scientific research –** for example to study the fate and transport of contaminants and evaluate the efficiency of remediation systems.

As already presented in U1., sample is a portion of a system (environment), representative for the sampling place and moment. Samples are complex homogenous systems (solutions) or heterogeneous ones. Rarely we analyse a mono-component sample, in most of the cases, the real samples are multicomponent systems, consisting in compounds that are present as major component, or as trace components. Considering complexity of an environmental sample it is obvious why the characterisation of these samples is a complete and rigorous process, requiring minimal initial knowledge about the sampling site and composition.

Some delimitation of terms should be mentioned:

- *component/compound/substance* is a portion of the matter delimited by a certain elemental composition and having attributed a chemical formula;
- *analyte* represents the compound of interest that is subject of analysis;
- *analytes* are subject of *determination*;
- *samples* are subject of *analysis*.

Some typical possible polluting compounds that differ when air, water or soil are monitored, are given in in Table 1.

Table 1. Typical compounds present in air, water and soil.

Compounds	Air	Water	Soil
inorganic gases	O_2, CO_2, CO, SO_x, NO_x, Cl_2, H_2S, HCl	dissolved oxygen (DO)	
anions	nitrite, nitrate, sulphide, chloride, formate, acetate…		
heavy metals	very rare	Cu^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Hg^{2+}, Fe^{2+}	
VOCs(1)	VOCs	rare	very rare
POPs(2)	PAHs(3), PCBs(4), pesticides, explosives		
other	particulate matter	detergents	detergents, humic / fulvic acids

(1) Volatile Organic Compounds; (2) Persistent Organic Pollutants; (3) Polyaromatic Hydrocarbons; (4) Polychlorinated Biphenyls

The table is not presenting the regulated monitoring parameters, but some of the expected groups of compounds, according to the diversity criteria: aggregation status, sample nature and compounds origin, composition and hydrophobicity. To be noticed that the compounds possible to be found in water and soil samples are almost similar, due to the so called "soil solution" that contains most of the water-soluble substances dispersed in the soil interstitial zones.

2. ENVIRONMENTAL SAMPLING

Sampling is the generic term consisting in two distinguished groups of operations:

- *pre laboratory operations* – consisting in (i) on-site sample up-take/collection (usually also called "sampling"), (ii) on-site conservation, (iii) transportation and storage;
- *in laboratory* operations – consisting in sample pre-treatment and preparation for analysis; these include separation, purification, concentration, or other operations.

2.1. SAMPLING

For the compounds that could suffer considerable transformations during the transport to the laboratory, samples should be analysed on-site. For these measurements, adequate equipment, mainly based on selective sensors and reagents kits are available. Although the available equipment is not enough sensitive and precise for trace analysis, on-site measurements information is very valuable and aimed complete the sample final characterisation.

Table 2. presents the seven steps of sample's life, from sampling planning to sample discharge, when sample is expired or when is no longer needed or valid. The table also enumerates some related questions to take into account, in order to go through the seven stages of sample's life.

Table 2. Seven steps of sample's life

7 steps of sample's life	**Related aspects**
1. a sampling is planned	• when to take samples • how often samples will be taken • what samples to collect (air, water, soil) • who to take sample (sample custody)
2. sampling points are identified	• where to take samples

3. samples are collected (up-taken)	• how to collect samples • how many samples to take • how much sample is needed
4. samples are transferred to the laboratory	• how to preserve samples • how long the sample will be stable
5. samples are prepared and analysed	• what property to analyse (physical, chemical, biological)
6. samples are transformed in chemical data point	
7. samples expire and are discharged	

2.1.1. IMPORTANCE OF SAMPLING

Sampling is a very importance activity, taking into account that if the samples are not properly collected or if they are not representative of the place and time of sampling, the analytical data obtained on those samples will not be reliable, data will correspond to other evidence.

Sampling depends on the complexity and quantity of the samples and the analytes, as well as on the laboratory quality requirements: available standards for analytical methods to be used at the measurement stage, equipment, reagents and reference materials, or specialized personnel. Any information about the **sample history** will be useful for optimal choice of the analytical method.

2.1.2. ENVIRONMENTAL SAMPLES PRESERVATION AND STORAGE

For the compounds that are either stable or can be preserved during the pre-treatment period, conservation procedure is used. From the sampling site, conserved samples are transported to the laboratory and stored. These stages and operations are done according to standards (if available) and all the information should be carefully registered on samples labels and standard forms, to be further used for the final result formulation.

Samples labels containing information about:

- name of the person performing the sampling;
- date, hour, site and place of sample up-take

Samples are accompanied by a standard form containing information about:

- sampling aim and objectives;
- type of further analysis;
- type and status of the sampling container;
- observations about the extent of pollution and pollution sources;
- meteorological information;
- sample conservation conditions.

Preservation and storage of environmental samples, are pre-laboratory stages, aiming to avoid physical or chemical processes (Table 3.) that might occur during the transportation of the samples to the laboratory.

Table 3. Environmental samples preservation and storage

Avoided processes		**Sample preservation / storage**
physical	volatilization, diffusion, adsorption, absorption	• refrigeration / cooling • choosing adequate storage container
chemical / biochemical	microbial degradation, chemical reactions, photochemical reactions	• refrigeration / cooling • choosing adequate storage container • adding chemical reagents (preservatives)

Physical processes such as volatilization, diffusion, absorption or adsorption are avoided by cooling and by choosing the appropriate sampling and storage containers.

Chemical and biochemical processes, as well as microbial degradation, chemical or photochemical reactions, are also avoided by cooling and choosing the appropriate recipients, additionally by adding chemical reagents, so-called preservatives. The reagents used as preservatives will react with certain compounds present in the sample, this being an accepted practiced as long as the chemical reaction is complete, qualitatively and quantitatively controlled. These chemical processes will be taken into account during the calculations that will lead to express the final result.

In Figure 2. are presented several samples collected for environmental analysis, stored in different containers, like plastic flasks or bags.

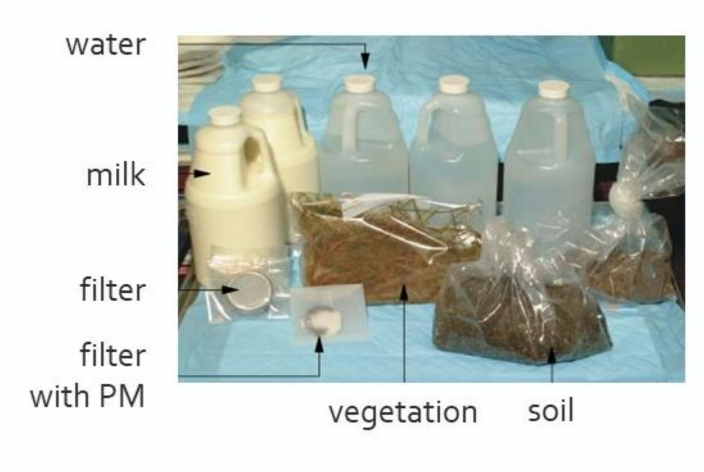

Figure 2. Examples of samples collected for environmental analysis.

Table 4. gives examples of preservation of the environmental samples, what reagents are used and which degradative processes can be avoided for certain compounds of interest, of inorganic or organic origin. For example:

- in order to avoid the precipitation of metals in the form of their oxides or hydroxides, nitric acid is added, which forms soluble metal nitrates;
- to avoid cyanide reaction with chlorine, ascorbic acid, known as vitamin C, is added;
- to avoid the volatilization of volatile organic compounds, complete filling of the sampling container is required;
- sampling in a dark container is used to avoid photodegradation of the petroleum products.

Table 4. Environmental samples preservation

Analyte	**Avoided processes**	**Sample preservation**
metals	oxides / hydroxides precipitation	HNO_3 (pH<2)
NH_3	volatilization	H_2SO_4 (pH<2)
H_2S and sulphides	volatilization	Zn acetate and NaOH (pH>9)
cyanide	volatilization	NaOH (pH>9)

	chemical reaction with Cl_2	ascorbic acid
dissolved organic compounds	chemical reaction with Cl_2	sodium thiosulfate
phthalic ester	diffusion through plastics	glass flasks or Teflon
VOCs*	volatilization	complete filling of the flasks
dissolved oxygen	introduction of oxygen from air	complete filling of the flasks
PAHs**	photochemical degradation	flasks of dark glass
petroleum products	adsorption on plastics	glass flasks

***** VOCs – volatile organic compounds; ** PAHs – polycyclic aromatic hydrocarbons

2.2. SAMPLE PRE-TREATMENT / PREPARATION

Sample pre-treatment / preparation is the second group of operations, as pre-analysis stage, to be performed on the samples, in the laboratory. The purpose of sample preparation is to bring the samples in a measurable form.

Sample preparation involves operations aiming both the sample and the analyte:

- operations affecting the sample:
    - weighting, drying, sieving – used to homogenize sample or remove moisture;
    - changing sample phase (liquid) – adequate for analytical instruments;
- operations affecting the analyte:
    - increasing /decreasing analyte concentration (concentration/dilution) – depends on the analyte concentration in the sample;
    - dissolution – is based on the analytes solubility properties; sometimes dissolution is also based on chemical reactions (chemical dissolution);
    - removing interfering compounds – analyte separation / purification from sample matrix; is done in order to distinguish the analyte(s) of interest from the rest of the compounds, co-existing in the sample; these “impurities” might introduce interfering information, similar to the analyte(s) of interest, thus contributing with errors and uncertainty to the final result;

- releasing analyte of interest from sample matrix – increase the detector response;
- modifying the analyte chemical structure – chemical derivatization that consists in the analytes chemical transformation in a new chemical compound, adequate to the measurement and/or detection systems.

Taking into account the physical or chemical modifications occurring during the sample pre-treatment, either simple, or more complex operations, Table 5. gives examples of such sample preparation operations.

Table 5. Sample preparation operations.

Physical operation		**Chemical operations**
Simple	**Complex**	
• weighing • drying • concentration • dilution	• dissolution • distillation • filtration • ad/absorption • extraction	• chemical dissolution • precipitation • ionic exchange • chelation • chemical derivatization

Separation method used in the sample pre-treatment

Most of the operations used in sample pre-treatment are based on separation processes and there are several modes to classify and group them. The classification we propose is based on different phase equilibrium:

– *gas–liquid equilibrium*: absorption, extraction, distillation;
– *gas–solid equilibrium*: adsorption, extraction;
– *liquid–liquid equilibrium:* liquid-liquid extraction techniques;
– *liquid–solid equilibrium*: filtration, centrifugation, ad/absorption, solid phase extraction (solid phase microextraction, solvent extraction, Soxhlet extraction, sonication extraction, microwave assisted extraction);

All these processes are contributing to the separation of some specific analytes from the rest of the matrix. The separation operations are nowadays developing in terms of method (principle) and technique (equipment) because analysts realised the importance of the sampling (including the pre-treatment phase) to the measurements and data processing. Only an adequate pre-treated sample could ensure a limited uncertainty of the final result.

From all the possible polluting compounds given in Table 1., we will further present two main classes of pollutants, considered of interest for environmental monitoring, with examples of typical pre-treatment procedures:

1. heavy metals cation, from the inorganic pollutants class;
2. organic compounds, grouped according to their specific properties (VOCs, POPs), specific composition (PAHs, PCBs), or specific use (pesticides, detergents).

2.2.1. SAMPLE PREPARATION FOR HEAVY METALS ANALYSIS

Sampling for heavy metals analysis is based on a very simple principle: to bringing the cations in a *soluble, stable and measurable form*:

a. either as monoatomic cations (Cu^{2+}, Fe^{2+}, etc);
b. or as metallic compounds (inorganic or organic), where metallic atoms are linked to another group of atoms, forming different measurable species – ions or neutral molecules.

Pre-Treatment Techniques Used for Liquid Samples

In order to solubilise the heavy metals, *ion exchange, precipitation, or chelation* are used, transforming the compound of interest in an extractible form, followed by the extraction of this. As soon as the soluble and extractible forms of the heavy metals are obtained, their solutions are ready for chemical analysis.

Among the extraction techniques used for heavy metals preparation for analysis from liquid samples the following are mentioned:

- **liquid-liquid extraction (LLE**) – with solvents of different polarities;
- **solid phase extraction (SPE)** – consisting of metal species retention / adsorption on an adequate sorbent, followed by the desorption with a suitable organic solvent or an inorganic one, like water also is.

Pre-Treatment Techniques Used for Solid Samples

Pre-treatment of samples for the analysis of heavy metals from solid samples is carried out by simply dissolution in water (cold or hot water), mineralization or by digestion. **Mineralization** consists of transforming inorganic and organic species into simple, inorganic (mineral) compounds.

The following **digestion** techniques are available for sample preparation aiming heavy metals analysis:

- acid digestion – with strong acids or strong acid mixtures with a powerful oxidant (HCl, HF, HNO_3, royal water, H_2SO_4 with H_2O_2);
- alkaline digestion – with NaOH or KOH, especially when the heavy metals of interest have amphoteric character (Cr, Mn);
- disaggregation by melting with fondants (NaOH or $K_2S_2O_8$) followed by the dissolution of the compounds obtained;
- calcination at high temperature – followed by the dissolution of the compounds resulting in the calcination ash, in the above mentioned acidic or alkaline conditions;
- digestion at high pressure – in digestion bombs;
- microwave digestion – combines the pressure effect with the temperature one.

2.2.2. SAMPLE PREPARATION FOR ORGANIC POLLUTANTS ANALYSIS

The pre-treatment of the samples for the organic compounds analysis is based on a similar principle with those used for heavy metals analysis: to extract the organic compound from the sample matrix in a soluble and stable form. This preparation stage depends on the state of aggregation of the sample matrix: liquid or solid.

Due to the more complex structures of the organic compounds (compared with the inorganic ones), the extraction techniques used in the pre-treatment stage are based on different physical properties of the analytes: phase transformations; absorptive properties; solubility in polar or non-polar solvents.

Organic Pollutants Extraction from Liquid Samples

Volatile organic compounds (VOC) are separated by static headspace extraction (extraction in saturated vapours) or by purge and trap extraction (vaporisation followed by condensation). Semi-volatile organic compounds (SVOC) and non-volatile organic compounds (NVOC) are separated into two stages: the first stage is aimed at obtaining a homogeneous liquid and is followed by the extraction step.

For the separation of SVOC and NVOC pollutants from liquid samples are used:
1. liquid-liquid extraction (LLE)
2. solid phase extraction (SPE) and solid phase microextraction (SPME)

Organic Pollutants Extraction from Solid Samples

For the semi-volatile and non-volatile organic compounds extraction from solid samples, the following techniques are more often applicable:

- Soxhlet (automated) extraction,
- accelerated solvents extraction (ASE),
- ultrasonic solvent extraction (USE),
- pressured solvent extraction (PSE),
- microwave assisted extraction (MAE).

As a specific application we have exemplified techniques for the extraction of pesticides from liquid or solid samples. In Table 6. are presented the extraction techniques, with corresponding applications depending on the aggregation state of the compounds, respectively of the sample. Of interest might also be some cost or technical information (such as extraction time).

Table 6. Examples of pesticides extraction from liquid and solid environmental samples.

Extraction technique	**Applications**	**Costs**	**Extraction time**
LLE	VOC, SVOC, NVOC compounds in liquid samples	low	1 h
SPE		medium	30 min
SPME		low	30 min
Soxhlet	SVOC, NVOC compounds in solid samples	low	12–48 h
USE		medium	15–30 min
MAE		medium	15 min
ASE (PSE)		high	20–30 min

Based on the complexity of the sample, the analyst will choose the most adequate pre-treatment operations, unless they are subject of standardisation, considering different selection criteria based on performance characteristics: selectivity; specificity; precision and accuracy; reproducibility; recuperation.

Particular information for air, water or soil sampling and sample pre-treatment will be presented in Topic 6.4.

3. ENVIRONMENTAL ANALYSIS

Most of the environmental analyses are regulated by standards, that are subject to reviewing from time to time, depending on the analytical methods and equipment development at the time. Moreover, sometimes the sample might be more complex than the available standard. Therefore, the aim of the following paragraphs is to present the *basic principles of the analytical methods and not the European or other international environmental standards*. We consider that understanding the principles of the analytical methods and techniques will contribute to the adequate use of them, to the correlation of all the available information.

Analytical chemistry is the science that studies and uses methods and instruments developed to separate, identify, and quantify the composition of matter/sample.

Chemical analysis represents a sum of on-site or in-lab operations performed to give qualitative and quantitative information about the composition of a complex sample. Chemical analysis of a sample consists in characterizing it from the point of view of its chemical composition and, in principle, is based on a measurable property of the solute (analyte), sometimes of the solution (sample).

3.1. ANALYTICAL METHODS PRINCIPLES

Analytical methods principles are based on a measurable property (P) of the analyte (A): mass, volume, thermal, electrical, or optical property.

The development of the **analytical methods**, in terms of mechanisms or equipment, allowed new **analytical techniques** development of the same method, meaning that the analytical technique is defined by same measurable property. Analytical techniques belong the same method, having the same principle, but developed based on different measuring mechanisms.

The determination of the chemical composition of a sample imposes two types of information to be acquired: *qualitative information* (qualitative analysis, or identification); *quantitative information* (quantitative analysis, or quantification). Quantitative analysis means to find the *measurable property (P)* that can be related as a function of the *analyte concentration* (C_A), based on physical-chemical laws: $P=f(C_A)$. No reliable quantitative information can be determined without a correct and complete qualitative characterization of the sample.

3.2. CLASSIFICATION OF THE ENVIRONMENTAL ANALYTICAL METHODS

Analytical methods can be grouped into two categories:

- **classical analytical methods** – also called wet chemical methods;
- **modern or instrumental analytical methods**; analytes are subject of physical transformation during the measurements, thus being non-destructive methods; these methods use calibration curves for quantitative determinations obtained on standard solutions P=f (CA).

The chemical methods (classical) were firstly developed, being dependant on technology development in time, and are based on chemical reactions that occur between the analyte and specific reagents, therefore they are destructive methods. Instrumental analytical methods are more recently developed, based on physical transformation of the analyte, therefore are non-destructive.

Sometimes a combination of physical and chemical transformation is required and the methods are thus called physical-chemical ones. Usually, a chemical pre-treatment of the analyte occurs (e.g. a derivatisation), followed by a physical property transformation.

In Table 7. are presented the main analytical methods and related techniques, with information on the measured property, of which the most commonly used methods will be further presented: gravimetric, titrimetric, spectrometric and chromatographic techniques.

Table 7. Methods and techniques used for environmental analysis.

Methods	**Techniques**	**Measured property**
Gravimetric (1), (2)	• electro-gravimetry • thermo-gravimetry	mass of pure analyte or of a mixture
Titrimetric (1), (2)	• acid-base • redox • precipitation • complexation	volume of the solution of a standard reagent that react with the analyte
Electrochemical methods (3), (4)	• voltammetry • potentiometry • conductometry	electrical properties of the analyte solutions: potential, electromotive force, conductibility

Spectrometry (3), (4)	• atomic / molecular spectrometry • absorption / emission spectrometry	wavelength and intensity of electromagnetic radiation emitted or absorbed by the analyte
Chromatography (3), (4)	• gas chromatography • liquid chromatography	repartition of the analyte between two non-miscible phases (mobile and stationary phase)

(1) – quantitative analysis; (2) – pollutants concentration at major, minor level, sometimes traces; (3) – qualitative and quantitative analysis; (4) – pollutants concentration at major, minor or traces level.

3.2.1. WET CHEMICAL METHODS AND APPLICATIONS IN ENVIRONMENTAL ANALYSIS

Gravimetry is a chemical classical method, which *principle* is based on measuring the mass of an analyte itself, or in most of the cases the mass of a product, obtained during a chemical reaction.

The method is applied for environmental samples, for the determination of moisture, or solids, oils and grease content.

Titrimetry is aslo a chemical classical method, which *principle* is based on the reaction between the analyte and a specific reagent (acid/base, redox, precipitation or complexing reagent), measuring the volume of a standard solutions of a compound involved in the titration reaction.

Titrimetric techniques are used for environmental analysis, like:

- acid-base titration for acidity, alkalinity determination;
- redox titration for residual chlorine and sulphide determination;
- precipitation titration for chloride determination;
- complexation titration for cyanide determination.

3.2.2. SPECTROMETRIC TECHNIQUES AND APPLICATIONS IN ENVIRONMENTAL ANALYSIS

Table 7. shows some spectrometric techniques with information about the principle of the method and examples of applications in environmental analyses.

Spectrometric techniques may be of adsorption or emission, for atomic or molecular determinations. This wide range of principles allows the use of spectrometric techniques to determine a large number of pollutants in environmental samples. For example, atomic absorption spectrometry is used to determine heavy metals in trace analysis, while molecular adsorption spectrometry allows the determination inorganic and unsaturated organic compounds (Table 8.).

3.2.3. CHROMATOGRAPHY TECHNIQUES AND APPLICATIONS IN ENVIRONMENTAL ANALYSIS

Another group of analytical methods used in environmental analysis is chromatography, able to deliver complex information: *separation, qualitative, quantitative, as well as structural.* The *principle* of chromatography is the differentiated distribution of analytes between two immiscible phases between them, a mobile phase (a gas or a liquid) and a stationary one (a liquid or a solid).

Table 8. Spectrometric techniques and their applications in environmental analyses.

Technique	**Acronym**	**Principle**	**Applications**
Atomic emission spectrometry	AES ICP-AES	atomic emission in arc, in flame; atomic emission in plasma	metals and non-metals (traces)
Fluorescence atomic spectrometry		fluorescence atomic emission	Hg, non-metal hydrides (traces)
Fluorimetry		molecular emission	PAHs
Atomic absorption spectrometry	AAS	atomic absorption	metals and non-metals (traces)
UV-VIS spectrometry	UV-VIS	molecular absorption	inorganic species and unsaturated organic compounds
IR spectrometry	IR	molecular absorption	gases, solutions, or solids of inorganic or organic compounds

Mass spectrometry	MS	molecular ionization and fragmentation	identification and structural analysis of organic compounds

Gas chromatography, liquid chromatography and ion chromatography have found applications for the separation, qualitative and quantitative determination of pollutants from environmental samples (Table 9.). For example, gas chromatography is used for analyses of gaseous pollutants, volatile or semi-volatile organic compounds.

Table 9. Chromatographic techniques and their applications in environmental analyses.

Chromatographic technique	Applications
Gas chromatography (GC)	gases, VOCs, SVOCs
Liquid chromatography (LC)	SVOCs, NVOCs (pesticides, PAHs, PCBs)
Ion chromatography (IC)	ionic species and polar molecules

Chromatographic techniques are suitable hyphenated techniques, coupling with mass spectrometry (MS), which plays an important role in identifying (structural information) of the organic compounds:

- GC-MS; GC–MS-MS;
- LC-MS; LC–MS-MS.

3.2.4. STAGES IN ENVIRONMENTAL MEASUREMENTS REQUIRING METHODOLOGY

For environmental analysis, usually, standardised sampling, sample preparation and analytical methods are available, but sometimes the sample might be more complex or inadequate for one particular standard. Therefore, all these operations are subject of method development (Figure 3.).

Analytical methods registered a long time for method development, carried out by chemical analysts, in specialised research of professional laboratories. Later on, considering the importance of the sampling and sample preparation importance on the total uncertainty of the final result, new methods and techniques related to these analytical stages were also developed.

When no standard is available, or when even if available the standard is not applicable in a certain laboratory, the analyst is in the position to choose the most adequate methods and techniques, for both sample preparation and analysis. The choice of these techniques should take into account different evaluation criteria: depending on the sample; depending on the methods performances and validation; depending on the analytical laboratory.

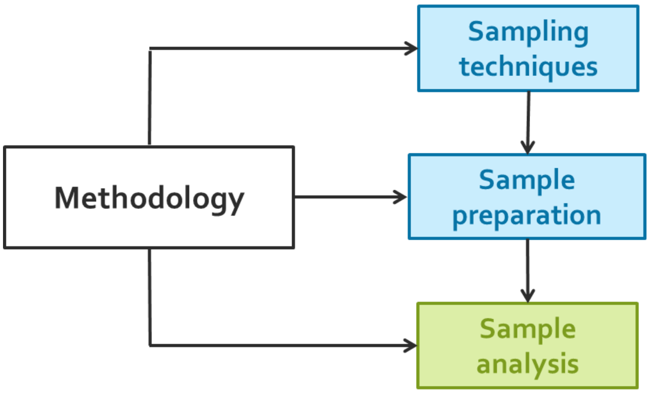

Figure 3. Stages in analytical process requiring method development.

3.3. QUALITY REQUIREMENTS FOR ENVIRONMENTAL MEASUREMENTS

Considering the execution stages of the monitoring flow, Figure 4. is presenting the entire analytical process that is subject of quality assurance and quality control (QA/QC).

Modern society is based on measurements. 40% of EU directives are referring to measurements, in technology, commerce, regulations, confirming the statement. Modern society requires quality in chemical measurement results, in such a way to make them accessible everywhere. There are two ways to ensure the quality of chemical measurements: (1) applying the quality management systems and accreditation (ISO/IEC 17025, 1999); (2) applying the principles of measurement science (metrology) to chemical measurements (metrology in chemistry – MiC). Thus, MiC was developed from the need to compare and have a uniform system to report the results.

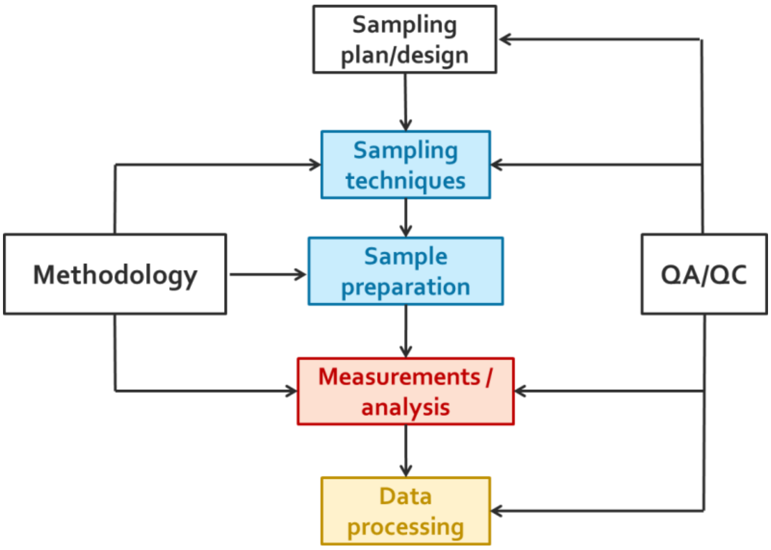

Figure 4. Analytical process requiring quality assurance and quality control.

In order to ensure the quality in chemical measurement results, in 1999 was launched the ISO/IEC 17025 accreditation standard (last version being from 2017), designed as guidance for general requirements for the competence to carry out tests and/or calibrations, including sampling, with standard methods, non-standard methods, or even laboratory-developed methods. ISO/IEC 17025 is based on the following aims:

- to provide a basis to be used by accreditation bodies when assessing the competence of laboratories;
- to establish general requirements for demonstrating laboratory compliance to perform specific tests or calibrations;
- to assist in the development and implementation of a laboratory’s quality system.

Accreditation allows the laboratory to demonstrate that the results are defensible to a recognized standard and do not change when either laboratory personnel or circumstances are changing. ISO/IEC 17025 is applicable to any organization performing tests and/or calibrations. It is used by: (i) laboratories accredited or in accreditation process; (ii) accreditation bodies; (iii) regulatory authorities.

In order to obtain the accreditation, the laboratory has to provide information related to the origin of method (standard or no-standard), comparison with the standard methods they replace (if applicable) as well as the validation of data procedure.

3.4. METROLOGY IN CHEMISTRY

Metrology in chemistry is assuming that chemical analysis is based on sampling and measurements, both stages contributing to the measurement uncertainty. Metrological principles are important for all analysts who deal with chemical measurements, the most relevant topics of interest for MiC being, of which *method validation and uncertainty estimation* will be further presented in this unit:

a. establishing *traceability* of the results of the measurements,
b. using *validated methods* of analysis,
c. estimating the *uncertainty of measurement results*,
d. using *certified reference materials* (CRMs) and
e. participation in *proficiency testing* (PT) schemes & *interlaboratory comparison* (ILCs).

EURACHEM is a network of organisations in Europe having the objective of establishing a system for the international traceability of chemical measurements and the promotion of good quality practices. EURACHEM has published a range of guides on quality and accreditation issues in analytical measurement, for all topics of interest above mentioned (a – e). All Guides are available from the EURACHEM website.

3.4.1. METHOD VALIDATION

According to the EURACHEM Guide (2014), method validation is the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires. Validation is also considered to give the confirmation by examination and provision of objective evidence that the particular requirements of a specified intended use are fulfilled, or **fit to purpose**. A method should be validated whenever a change in the analytical process occurs, and the laboratory has to demonstrate that its performance parameters are adequate for its use for a particular analytical problem, meaning:

- new method was developed;
- the existing method was revised;
- method is changing with time;
- method is used in a different laboratory, or with different analysts or different instrumentation;
- the method is equivalent with another one, for example a standard one.

To validate a method is compulsory to demonstrate the following method performance criteria: specificity / selectivity, precision, trueness, linearity and linearity range, limit of detection (LOD), limit of quantification (LOQ), and ruggedness / robustness. As presented in Table 10. not all the method performance parameters are required for any validation procedure. They depend on the analytical purpose: identification tests; impurity tests; assay tests.

Table 10. Method performance criteria dependent on the analytical purpose.

Method-performance parameter	**Identification test**	**Impurity test**		**Assay test**
		Limit impurity test	**Quantitative impurity test**	
selectivity and specificity	+	+	+	+
trueness	-	-*	+	+
precision	-*	-	+	+
linearity and linear range	-*	-	+	+
limit of detection (LOD)	-*	+	-	-
limit of quantification (LOQ)	-*	-	+	-
robustness	+	+	+	+

* may be performed

Selectivity and specificity are measures which assess the reliability of measurements in the presence of interferences. A method is selective if it produces responses for a group of chemical entities or analytes which can be distinguished from each other, while the method is specific if it produces a response for a single analyte only.

Accuracy gives the degree of spread of data and is studied as two components: *precision and trueness*.

Trueness of a method is a measure of how close the mean of a set of results (produced by the method) is to the true value. Due to the fact that the true value is always not known, it is replaced by a reference value, and estimated by the mean value. Practical assessment of trueness relies on comparison of mean results from a method with reference values, mostly used is the recovery test (R), based on spiking a blank sample with a known quantity of the pure and stable compound of interest (reference value), and calculated with equation 1.

Precision of the method is a measure of how close results are to one another. Precision may be evaluated as: intra-laboratory repeatability precision; intra-laboratory reproducibility precision (or intermediate precision); inter-laboratory reproducibility precision. Usually, precision is expressed by measures such as standard deviation (s or SD), dispersion (s^2), or relative standard deviation (RSD%), according to the equations 2-3:

$$R = \frac{C_F - C_I}{C_A} 100 \qquad 1.$$

$$s = \sqrt{\frac{\sum_1^n (\overline{X} - X_i)^2}{n-1}} \qquad 2.$$

$$RSD = \frac{s}{\overline{x}} \cdot 100 \qquad 3.$$

where: C_F is the final concentration of the spiked sample; C_I is the initial concentration before spiking; C_A is the concentration of the added spiking solution; X_i are discrete measured values, $\overline{X}$ is the average value of *n* repeated measurements.

Linearity and linearity range give information about the ability of the method to produce signal response (measurable property) in a linear relationship to the analyte concentrations (or quantity). At the lower end of the concentration range the limiting factors are the values of the limits of detection and/or quantitation.

Limit of detection (LOD) represents the lowest content of the analyte that can be measured with reasonable statistical certainty. It gives a “qualitative” information.

Limit of quantification (LOQ), or **limit of quantitation**, represents the lowest content of an analyte that can be determined with acceptable accuracy under the stated conditions of the test. It gives “quantitative” information.

Robustness and ruggedness are measures of the ability of the method to produce unaffected results by variations interviewing either at the method parameter (robustness), or at the operation conditions (ruggedness).

3.4.2. UNCERTAINTY ESTIMATION

According to the EURACHEM Guide (2012), uncertainty (U) is a parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand (result). Uncertainty may be expressed as relative standard deviation (RSD) and confidence interval.

Uncertainty estimation **is required** whenever:

- a new procedure/method is introduced in the laboratory;
- an important process parameter is modified (equipment, sample type);
- a new method is validated;
- **not required** for daily analysis.

Table 11. presents the uncertainty sources, arising both from sampling and from analytical measurements.

Table 11. Uncertainty sources and estimation

Uncertainty contributions	Symbol	Uncertainty sources
Contribution of sampling	U_S	sampling, transporting, storage
Contribution of measurements	U_M	equipment, reagents purity, measurements conditions
Total uncertainty	U	$U = U_S + U_M$

There are two steps to follow in order to estimate the uncertainty:

1. identifying the uncertainty sources and build up the Ishikawa diagram;;
2. uncertainty calculation, considering the surces, that have the most relevant contribution to the total uncertainty.

The Ishikawa diagram may be separatelly built-up considering the uncertainty sources from sampling, from measurements on standard solutions (Figure 5.), or on real samples (Figure 6.). It was demonstrated that the sampling contribution to the total uncertainty is quite considerable, sometimes arriving up to 80% of the U value.

For the client, uncertainty estimation means that the final result (R) is announced as a sum between the average concentration (C) and the uncertainty contribution (equation 4).

$$R = C \pm U \qquad 4.$$

For the laboratory uncertainty estimation gives the degree of trust associated to the analytical procedure.

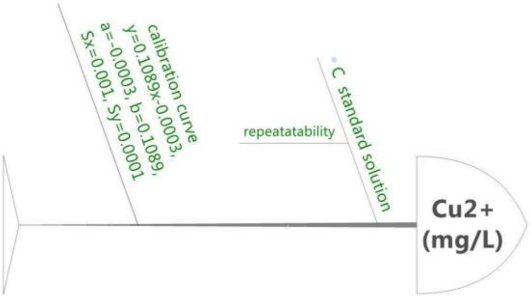

Figure 5. Ishikawa diagram for the uncertainty sources on standard solutions.

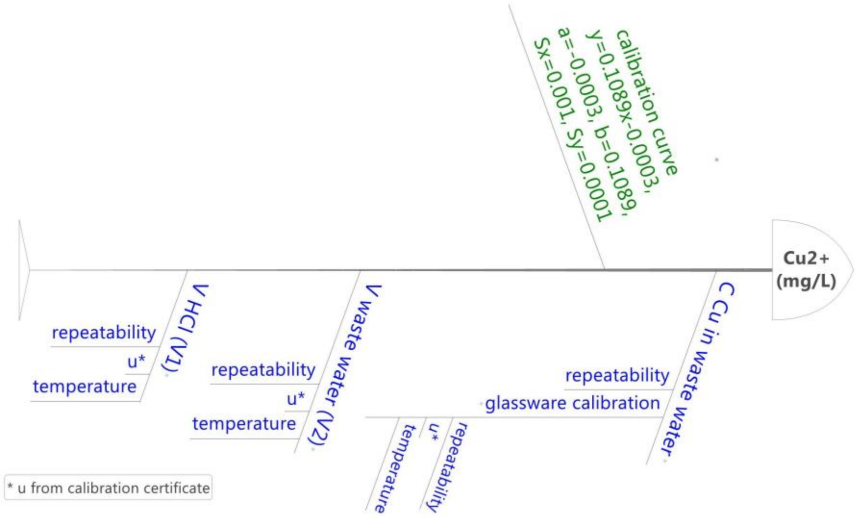

Figure 6. Ishikawa diagram for the uncertainty sources on wastewater samples.

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