Hort4EUGreen

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NUTRITIONAL QUALITY OF HORTICULTURAL PRODUCTS

Tricks and tips in analysis methods of nutritional quality of horticultural products

Volume 4

University of Agronomic Sciences and Veterinary Medicine of Bucharest
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CONTRIBUTORS

USAMV

Liliana BĂDULESCU
Monica BADEA
Violeta Alexandra ION
Oana - Crina BUJOR
Andreea BARBU
Aurora DOBRIN
Carmen CONSTANTIN

University of Agronomic Sciences and Veterinary Medicine of Bucharest

Editing:
Oana VENAT
Alexandra CORNEA

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"Enhancing practical skills of horticulture specialists to better address the demands of the European Green Deal"

Bucharest, 2025

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Introduction

The “Tricks and tips in analysis methods of nutritional quality of horticultural product”` Module is intended to provide some special hints to the students, especially the master students that will work directly in the laboratory and perform different analysis on nutritional quality of horticultural products.

The module consists of two units, one on tips for the selection of appropriate analysis methods and one on tricks and tips regarding the sample preparation, followed by annexes where tips for each major analysis methods are given, as ICP-MS equipment.

Learning outcome descriptors

By the end of the Module, the trainee should be able to:

General and transferable skills

1	Prepare a working station for specific analytical method
2	Work in the laboratory independently or with a minimal guidance where appropriate
3	Work in team with minimal guidance where appropriate
4	Show good laboratory skills
5	Demonstrate the capacity to understand the obtained results in order interpret the information from a variety of analytical methods

Knowledge, understanding and professional skills

1	Select the appropriate analytical method for determination of a certain parameter related to nutritional quality of fruits and vegetables
2	Be confident that the work is performed according to the protocol and the results are representative
3	Gain working knowledge in laboratory approach using different methods of characterization

Unit 4.1: Selection of appropriate analysis method

Aurora Dobrin, Carmen Constantin, Andreea Barbu, Oana-Crina Bujor, Violeta Alexandra Ion

Unit 4.1.1. What nutrients should be analyzed

Horticultural products analysis is the discipline dealing with the development, application, and study of analytical procedures for characterizing their properties and constituents. These analytical procedures are used to provide information about a wide variety of quality characteristics, including structure and physicochemical properties (size, color, shape), sensory attributes (flavor: taste and texture), nutritional composition, health benefits and shelf life. This information is critical to our rational for understanding the factors that determine the properties of horticultural products, to our ability to economically produce them which are consistently safe, nutritious and desirable as well as to consumers to make informed choices about their diet.

Nutritional quality of fruits and vegetables, either fresh or processed, is generally based on the chemical composition or physical characteristics or a combination of these two factors.

Horticultural products are analyzed by scientists working in all of the major sectors of the horticulture industry including manufacturers, processors, analytical service laboratories, government laboratories, and University research laboratories. The various purposes that horticultural products are analyzed are briefly discussed in this unit.

Government Regulations and Recommendations

Government regulations and recommendations were developed to maintain the general quality of the horticultural products supply, to ensure the horticultural products industry provides consumers with horticultural products that are wholesome and safe, to inform consumers about the nutritional composition of horticultural products so that they can make knowledgeable choices about their diet, to enable fair competition amongst companies, and to eliminate economic fraud.

Standards

Voluntary and mandatory standards relating to the composition, quality, inspection, and labeling of specific horticultural products are elaborated by government agencies. These regulations specify the type and amounts of ingredients that certain horticultural products must contain if they are to be called by a particular name on the horticultural products label. For example, fruits are graded according to their quality, from standard to excellent, according to their origin, tenderness, juiciness, flavor and appearance. There are clear definitions associated with these descriptors that products must conform to before they can be given the appropriate label.

Nutritional Labeling

Nutritional labels describe the nutrient content of the horticultural products (the total calorific value, total fat, saturated fat, cholesterol, sodium, carbohydrate, dietary fiber, sugars, protein, vitamins, calcium and iron) and can be used by consumers to plan a nutritious diet, to avoid the consumption of horticultural products components linked with health impact, and to encourage greater consumption of horticultural products that are beneficial to health.

Authenticity

There is important to use analytical techniques that can be applied to test the authenticity of certain nutrients, to ensure that consumers are not the victims of economic fraud and that competition among horticultural products manufacturers is fair. It is well known that the price of certain horticultural products is dictated by the quality of the ingredients that they contain. For example, a packet of premium coffee may claim that the coffee beans are from Columbia, or the label of an expensive wine may claim that it was produced in a certain region, using a certain type of grapes in a particular year. It is necessary to verify these claims.

Horticultural products Inspection and Grading

The government has a **Horticultural products Inspection and Grading Service** that routinely analyses the properties of horticultural products to ensure that they meet the appropriate laws and regulations. **National Sanitary Veterinary and Horticultural products Safety Authority (ANSVSA)** is responsible for the Program of Pesticide Residue Control in cooperation with the other two competent authorities. Also, ANSVSA has the responsibility for the elaboration and implementation of its own **National Program for the Control for Horticultural Products of Plant and Animal Origin**.

Hence, both government agencies and horticultural product manufacturers need analytical techniques to provide the appropriate information about their properties. The most important criteria for this type of test are often the accuracy of the measurements and the use of an official method. The government has recently carried out a survey of many of the official analytical techniques developed to analyze horticultural products, and has specified which techniques must be used to analyze certain components for labeling purposes. Techniques have been chosen which provide accurate and reliable results, but which are relatively simple and inexpensive to perform.

Horticultural products and horticultural products safety

One of the most important reasons for analyzing horticultural products from both the consumers and the manufacturers standpoint is to ensure that they are safe. It would be economically disastrous, as well as being rather unpleasant to consumers, if a horticultural products manufacturer sold a product that was harmful or toxic. It is therefore important that horticultural products manufacturers do everything they can to ensure that these harmful substances are not present, or that they are effectively eliminated before the horticultural products is consumed.

This can be achieved by following good manufacturing practice regulations specified by the government for specific horticultural products and by having analytical techniques that are capable of detecting harmful substances. In many situations it is important to use analytical techniques that have a high sensitivity, i.e., that can reliably detect low levels of harmful material. Horticultural product manufacturers and government laboratories routinely analyze these products to ensure that they do not contain harmful substances and that the production facility is operating correctly.

Quality control

The horticultural products industry is highly competitive and horticultural products manufacturers are continually trying to increase their market-share and profits. To do this they must ensure that their products are of higher quality, less expensive, and more desirable than their competitors, whilst ensuring that they are safe and nutritious. To meet these rigorous standards horticultural products manufacturers, need analytical techniques to analyze horticultural materials before, during and after the manufacturing process to ensure that the final product meets the desired standards.

In a horticultural products factory one starts with a number of different raw materials, processes them in a certain manner (e.g. heat, cool, mix, dry), packages them for consumption and then stores them. The horticultural products is then transported to a warehouse or retailer where it is sold for consumption.

One of the most important concerns of the horticultural products manufacturer is to produce a final product that consistently has the same overall properties, i.e. appearance, texture, flavor and shelf life. When we purchase a particular horticultural products product, we expect its properties to be the same (or very similar) to previous times, and not to vary from purchase-to-purchase. Ideally, a horticultural products manufacture wants to take the raw ingredients, process them in a certain way and produce a product with specific desirable properties.

Unfortunately, the properties of the raw ingredients and the processing conditions vary from time to time which causes the properties of the final product to vary, often in an unpredictable way. How can horticultural products manufacturers control these variations? Firstly, they can understand the role that different ingredients and processing operations play in determining the final properties of horticultural productss, so that they can rationally control the manufacturing process to produce a final product with consistent properties.

Secondly, they can monitor the properties of horticultural productss during production to ensure that they are meeting the specified requirements, and if a problem is detected during the production process, appropriate actions can be taken to maintain final product quality.

Manufacturers measure the properties of incoming raw materials to ensure that they meet certain minimum standards of quality that have previously been defined by the manufacturer. If these standards are not met the manufacturer rejects the material. Even when a batch of raw materials has been accepted, variations in its properties might lead to changes in the properties of the final product. By analyzing the raw materials it is often possible to predict their subsequent behavior during processing so that the processing conditions can be altered to produce a final product with the desired properties.

For example, the color of potato chips depends on the concentration of reducing sugars in the potatoes that they are manufactured from: the higher the concentration, the browner the potato chips. Thus, it is necessary to have an analytical technique to measure the concentration of reducing sugars in the potatoes so that the frying conditions can be altered to produce the optimum colored potato chips.






4.1.2 How can we choose an analytical technique?

There are usually a number of different analytical techniques available to determine the nutritional value of fruit and vegetables. It is therefore necessary to select the most appropriate technique for the specific application.

The analytical technique selected depends on:

- the property to be measured;
- the type of matrix to be analyzed;
- the reason for carrying out the analysis.

Information about the various analytical procedures available can be obtained from a number of different sources. An analytical procedure may already be routinely used in the laboratory or company where you are working.

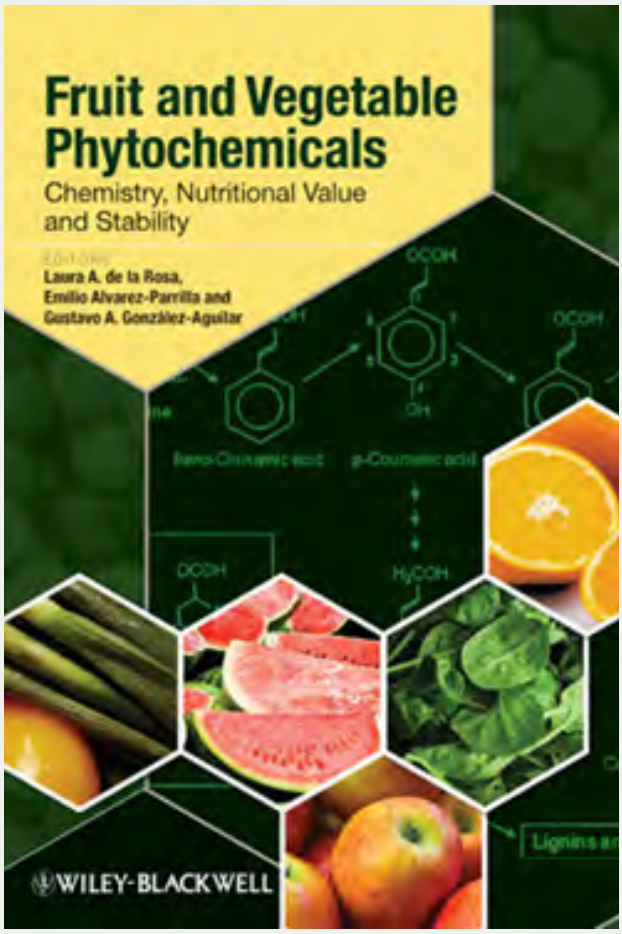
Alternatively, it may be possible to contact an expert who could recommend a certain technique, e.g., a **University Professor** or a **Consultant**. Often it is necessary to consult scientific and technical publications.

4.1.3 Appropriate selection of an information source

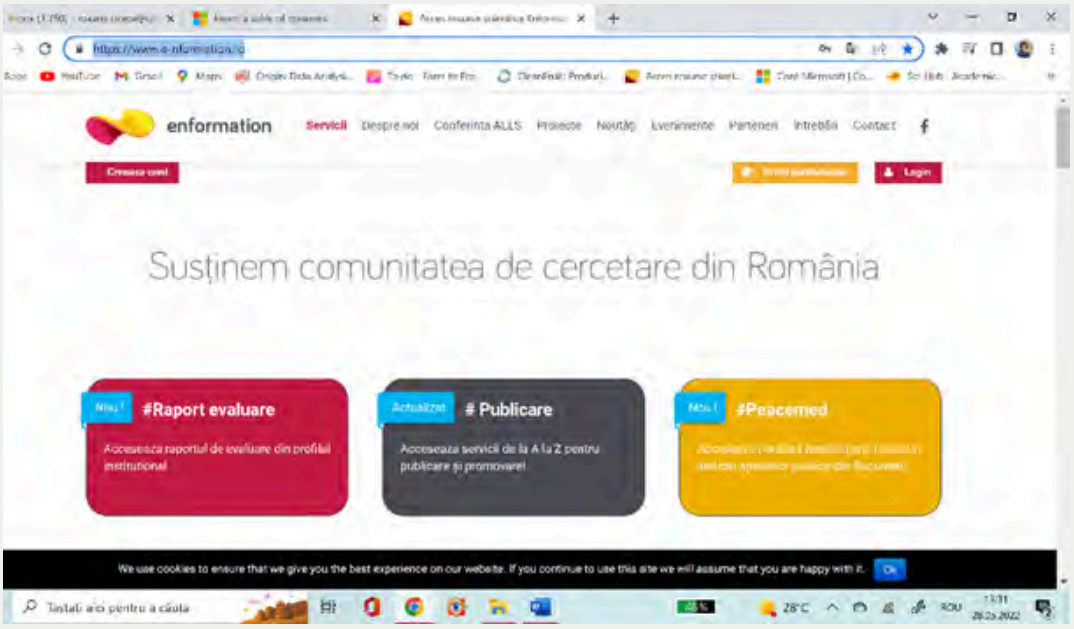
A. Books

Analysis books may provide a general overview of the various analytical procedures used to analyze fruits and vegetables properties or they may deal with specific components or physicochemical characteristics.

Consulting a general textbook on horticultural products analysis is usually the best place to begin to obtain an overview of the types of analytical procedures available for analyzing them and to critically determine their relative advantages and disadvantages.



A vast diversity of data is available on online data bases. Some links and hints about the use of such data bases are offered in Romania on the e-nformation platform (https://www.e-nformation.ro/), that offer access to the Web of Science, ScienceDirect Freedom Collection, CAB Abstracts, CAB Ebooks, CABI VETMED Resource, Elsevier Ebooks, Elsevier Emerald eBooks Collection, Nature Journals, Sage eBooks Collections, Scopus, Elsevier, SpringerLink Journals, Springer, Wiley Ebooks, De Gruyter ebooks. Also, different open data bases can be accessed.



The WHO (**World Health Organization**) has a section dedicated to Nutrition and food safety, where several data bases are included

https://www.who.int/teams/nutrition-and-food-safety/databases

USDA Food and Nutrient Databases provide the Infrastructure for Food and Nutrition Research, Policy, and Practice (Ahuja et al, 2013), at

https://fdc.nal.usda.gov/index.html

FAO (**Food and Agriculture organization of the United Nations**) also gives access to a database containing data about food consumption in many countries of the world

https://www.fao.org/gift-individual-food-consumption/data-and-indicator/en/

B. Tabulated Official Methods of Analysis

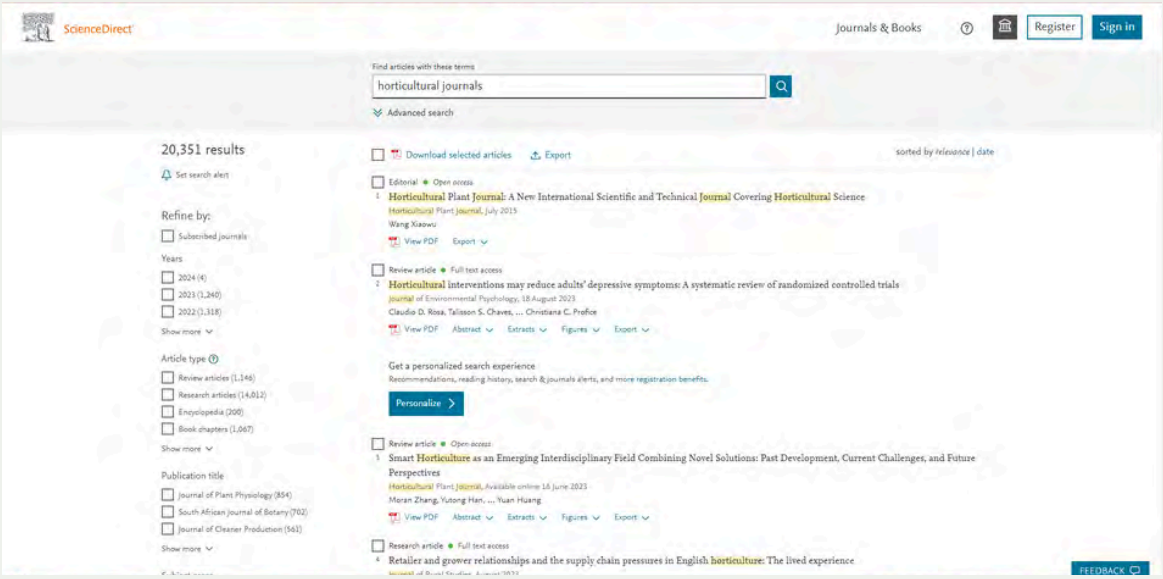
A number of scientific organizations have been setup to establish certain techniques as official methods, e.g. Association of the Official Analytical Chemists (AOAC), ISO - International Organization for Standardization, Romanian Standards Association (ASRO) and so on. Normally, a particular laboratory develops a new analytical procedure and proposes it as a new official method to one of the organizations. The method is then tested by a number of independent laboratories using the same analytical procedure and type of equipment stipulated in the original proposal.

Source: https://www.aoac.org/

Source: https://www.asro.ro

The results of these tests are collated and compared with expected values to ensure that the method gives reproducible and accurate results. After rigorous testing the procedure may be accepted, modified or rejected as an official method. Organizations publish volumes that contain the officially recognized test methods for a variety of different horticultural products components and horticultural product stuffs. It is possible to consult one of these official publications and ascertain whether a suitable analytical procedure already exists or can be modified for your particular application.

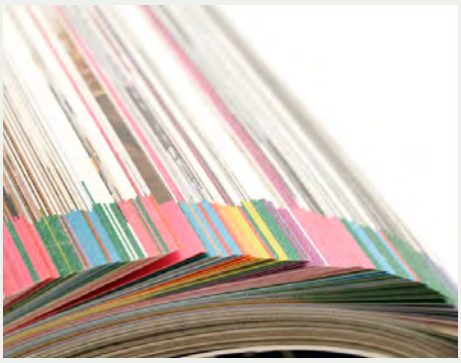
C. Journals



Source:https://www.sciencedirect.com/search?qs=horticultural%20journals

Analytical methods developed by other scientists are often reported in scientific journals, e.g., Journal of Horticultural products Science, Journal of Agriculture and Horticultural products Chemistry, Analytical Chemistry. Information about analytical methods in journals can often be obtained by searching computer databases of scientific publications available at libraries or on the Internet (e.g., Web of Science, ScienceDirect, Springer Link, MDPI etc.).

D. Equipment and Reagent Suppliers



Many companies that manufacture equipment and reagents used to analyze horticultural productss advertise their products in scientific journals, trade journals, trade directories, and the Internet.

These companies will send you literature that describes the principles and specifications of the equipment or test procedures that they are selling, which can be used to determine the advantages and limitations of each technique.

E. Internet

The Internet is an excellent source of information on the various analytical procedures available for analyzing horticultural products properties. University lecturers, book suppliers, scientific organizations, scientific journals, computer databases, and equipment and reagent suppliers post information on the web about horticultural products analysis techniques. This information can be accessed using appropriately selected keywords in an Internet search engine.

4.1.4 Developing a new technique

In some cases, there may be no suitable techniques available and so it is necessary to develop a new one. This must be done with great care so as to ensure that the technique gives accurate and reliable measurements. Confidence in the accuracy of the technique can be obtained by analyzing samples of known properties or by comparing the results of the new technique with those of well-established or official methods.

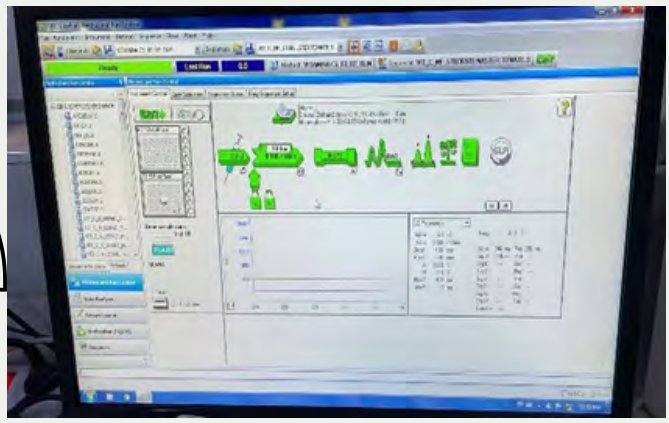
Most horticultural products contain a large number of different components, and therefore it is often necessary to distinguish the component being analyzed for ("the analyte") from the multitude of other components surrounding it ("the matrix").

One of the most important factors that must be considered when developing a new analytical technique is the way in which the analyte will be distinguished from the matrix.

Horticultural products components can be distinguished from each other according to differences in their molecular characteristics, physical properties and chemical reactions:

- Molecular characteristics: size, shape, polarity, electrical charge, interactions with radiation

- Physical properties: density, rheology, optical properties, electrical properties, phase transitions (melting point, boiling point)
- Chemical reactions: Specific chemical reactions between the component of interest and an added reagent



When developing an appropriate analytical technique that is specific for a particular component it is necessary to identify the molecular and physicochemical properties of the analyte that are sufficiently different from those of the components in the matrix. In some horticultural products it is possible to directly determine the analyte within the horticultural products matrix, but more often it is necessary to carry out a number of preparatory steps to isolate the analyte prior to carrying out the analysis.

For example, an analyte may be physically isolated from the matrix using one procedure and then analyzed using another procedure. In some situations there may be one or more components within a horticultural products that have very similar properties to the analyte.

If there are a number of alternative methods available for measuring a certain property of a horticultural products, the choice of a particular method will depend on which of the above criteria is most important.

For example, accuracy and use of an official method may be the most important criteria in a government laboratory which checks the validity of compositional or nutritional claims on horticultural products products, whereas speed and the ability to make nondestructive measurements may be more important for routine quality control in a factory where a large number of samples have to be analyzed rapidly.

These "interferents" may make it difficult to develop an analytical technique that is specific for the analyte. It may be necessary to remove these interfering substances prior to carrying out the analysis for the analyte, or to use an analytical procedure that can distinguish between substances with similar properties.

The **criteria** that are important in selecting a technique are listed below:

- **Selectivity** – Is your selected analytical method capable to differentiate between compounds?
- **Accuracy** – Does your selected analytical method can correctly identify the amount of the interested compounds?
- **Precision**; repeatability intra-laboratory (within laboratory), reproducibility inter-laboratory within laboratory and between laboratories) – Are you sure your analytical method does the same thing over and over again?
- **Limit of detection** – Is your selected method capable of identifying low amounts of the interested compounds?
- **Sensitivity** - Is your selected method capable of differentiate between different amounts of the interested compounds?
- **Practicability** and **applicability** under normal laboratory conditions – Can you actually apply the method in your laboratory? Do you have the necessary equipment, reagents and personal to implement the method?
- Other criteria which may be selected as required (e.g. cost, so on).

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Unit 4.2 Tricks and tips regarding sample preparation

Aurora Dobrin, Carmen Constantin,
Oana-Crina Bujor, Violeta Alexandra Ion

Sample preparation of horticultural products and agricultural products is highly dependent on the properties of the analyte and the nature of the sample matrix. For example, solid or semi-solid samples such as fruit, vegetables, tissue or soil need sample pre-treatment for efficient extraction of the analytes. The samples are prepared prior to performing the specific determination and is made by using adequate equipment (mills, bolters, drying ovens, etc.) to provide the granulation and moisture characteristics, necessary for each ordered set of analyses.

Usually, sample preparation can be performed in two basic steps:
(i) extraction of target analytes;
Carotenoid extracts from tomatoes
(ii) removal of interfering substances.

Remove traces of water

Horticultural products samples can be analysed directly only occasionally: in most cases they need a sample clean-up step, necessary to remove interfering substances.

Sometimes, this becomes a necessary step to make the analysis itself possible, as in the case of samples that need to be treated with derivatizing agents (e.g. methylation of free fatty acids prior to GC analysis).

For example, if considering the headspace evaluation of a coffee aroma, there is no need to perform any sample preparation procedure, because the objective of the investigation is the evaluation of a property possessed by the sample in its original form.

Chose the most suitable sample preparation techniques based on the following consideration:

Analyte

- What are the compounds structure and general properties?
- Does the analyte dissolves into the matrix?
- Does the analytes contain any ionic groups?
- Are the compounds unstable in acid or base?
- What is the derivatization method (for GC or LC analysis)
- What is the concentration of the analyte in the sample?

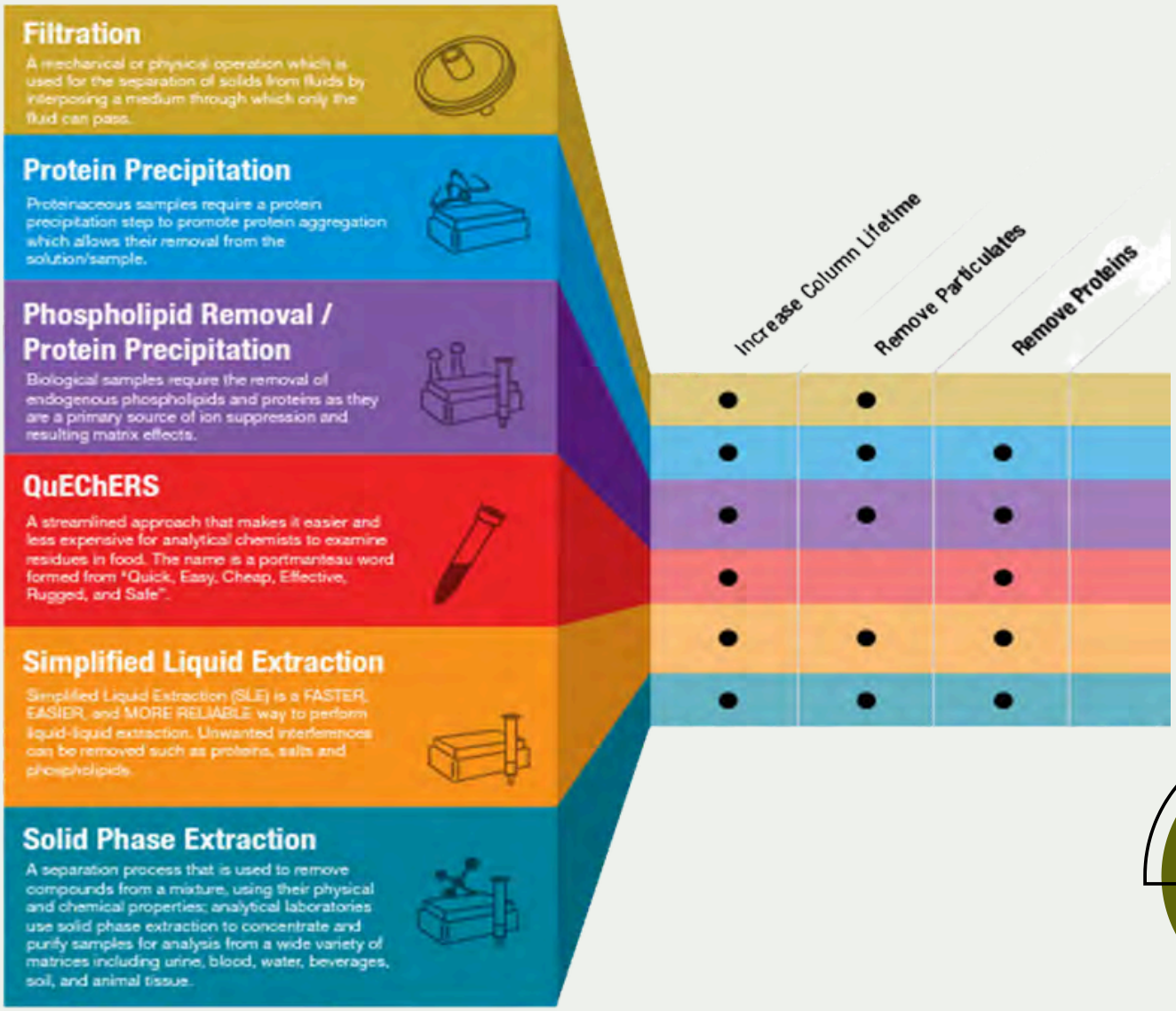
Matrix

- Is the matrix polar or nonpolar?
- Is the matrix high in organic or ionic content (do we need sample dilution?)
- Is any sample preatretment needed? (ex: pH adjustment)
- Do we need an internal standard?
- Do we need filtration or centrifugation?
- What are the major interferences?
- What are the differences between interferences and compounds?

Sample

- What is the sample volume and concentration?
- What are the number of samples to be processed?
- If the sample is solid, how do you process it and extract it?
- Stability considerations: do you need to keep the sample on ice, in the dark or add additives to prevent oxidation, enzymatic activity or non-specific biding?

Each of the sample preparation step has it trick from the simples steps (filtration) to the more complicated steps (SPE—solid phase extraction) in order to obtain the best results. We will continue with simple trick when using different types of sample preparation techniques.



Source: Phenomenex (https://www.phenomenex.com/Account/LogOn/form~2015sampleprepguidetool)

Filtration:

- Filter the samples with lower pore size (0.2 µm, 0.45 µm) to prolong column and detector life
- Check the compatibility of the filter material with the sample solvent

SPE- solid phase extraction

When developing a SPE method there are 2 factors to take in consideration:

Elution solvent

Acetonitrile and methanol are the usual elution solvents used for the SPE method.

Sample volume

which determines the sorbent volume. The elution volumes are specific to the chemical nature of the analyte being extracted, its concentration in the sample, the chemical nature of the eluting solvent and the bed mass used. An elution study should be conducted to determine the appropriate volume to be used.

When considering analyte type, the lowing tips should be used:

Water Soluble Target Compounds in Organic Solvent Matrix

- No equilibration of cartridges required. Condition the cartridge with the same organic solvent as the sample;
- Acceptable non-polar loading and washing solvents are: hexane, chloroform, methyl-t-butyl ether;
- Acceptable polar elution solvents are: tetrahydrofuran, ethyl acetate, isopropanol, acetonitrile and methanol as long as they are miscible with the loading/washing solvents.

Organic Soluble Target Compounds in Aqueous Matrix - Tips

- Condition with methanol. Equilibrate with water, modify the pH up if target molecules are slightly basic, down if slightly acidic;
- Acceptable polar loading and washing solvents are: Water, buffered water to adjust the pH, water with small amount (1-10% methanol);
- Acceptable organic elution solvents are: Methanol.

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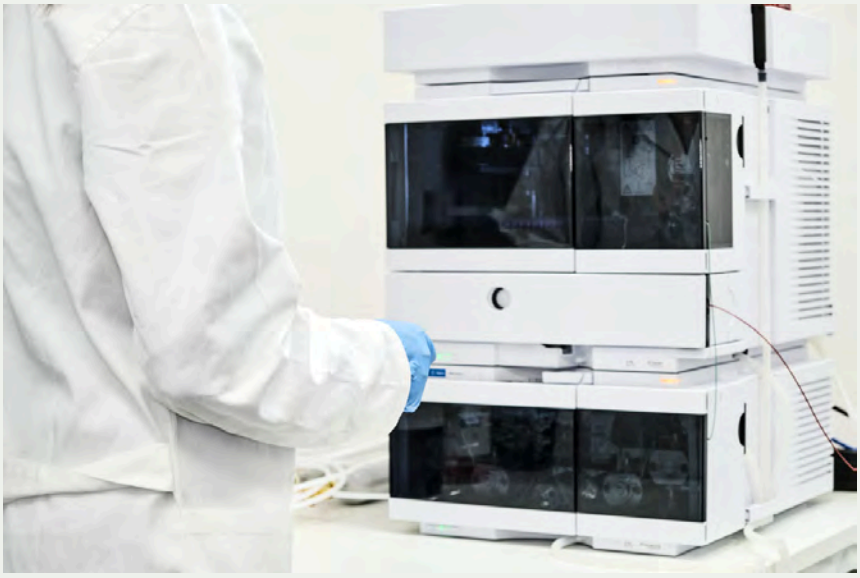
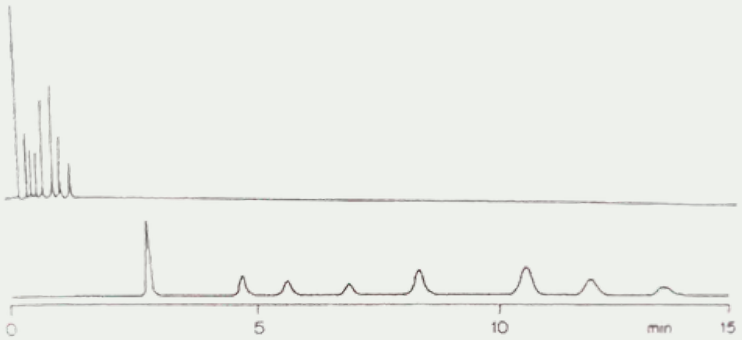


Appendix 4.1
Tricks and tips in HPLC analysis

Liliana Bădulescu, Oana-Crina Bujor, Violeta Alexandra Ion, Andreea Barbu, Monica Badea

Selection of appropriate column

The most important part of a chromatographic set up is the column. But even though it provides retention, the final separation also depends strongly on the mobile phase. Choosing the right chromatographic conditions matching optimum temperature, pH, solvent compatibility, working ranges of the column can minimize potential bleeding effects. Selection of best column and chromatographic conditions will lead to symmetrical peaks. Optimization of method conditions must be performed to select the most suited buffer, pH, and if necessary, additives. The use of a steep gradient can often yield a sharper peak than isocratic mode alone.



Comparison of performance between stationary phases of different particle diameter (Above: 6 cm x 4.6 cm, 3 µm ODS-Hypersil; Below: 20 cm x 4.6 cm, 10 µm ODS – Hypersil).
Source: Practical Hight Performance Liquid Chromatography, Fifth edition, Veronica R. Meyer, 2010 Jon Wiley & Sons, Ltd

Compounds	Separation mode
Monosaccharides	HILIC-Hydrophilic Interaction Chromatography
Disaccharides	LEX-Ligand Exchange Chromatography SEC-Size Exclusion Chromatography
Sugar alcohols	LEX-Ligand Exchange Chromatography HILIC-Hydrophilic Interaction Chromatography
Oligosaccharides	HILIC-Hydrophilic Interaction Chromatography LEX-Ligand Exchange Chromatography SEC-Size Exclusion Chromatography
Low molecular water soluble dietary fiber	SEC-Size Exclusion Chromatography
Polysaccharides	SEC -Size Exclusion Chromatography
Organic acids	RPC–Reverse Phase Chromatography IEX–Ion Exclusion Chromatography IC–Ion Chromatography
Water-solublevitamins	RPC- Reverse Phase Chromatography IEC - Ion Exchange Chromatography HILIC- Hydrophilic Interaction Chromatography
Fat-soluble vitamins	RPC - Reverse Phase chromatography NPC - Normal Phase chromatography SEC - Size Exclusion Chromatography
Amino acids	RPC- Reverse Phase chromatography IC - Ion Chromatographyv IEC - Ion Exchange Chromatography

Table 4.1 - 1. Column selection for separation of nutritional products in horticultural products
Souce: Guidelines for Shodex Column Selection : by Separation Mode, www.shodex.com

Selection of appropriate mobile phase

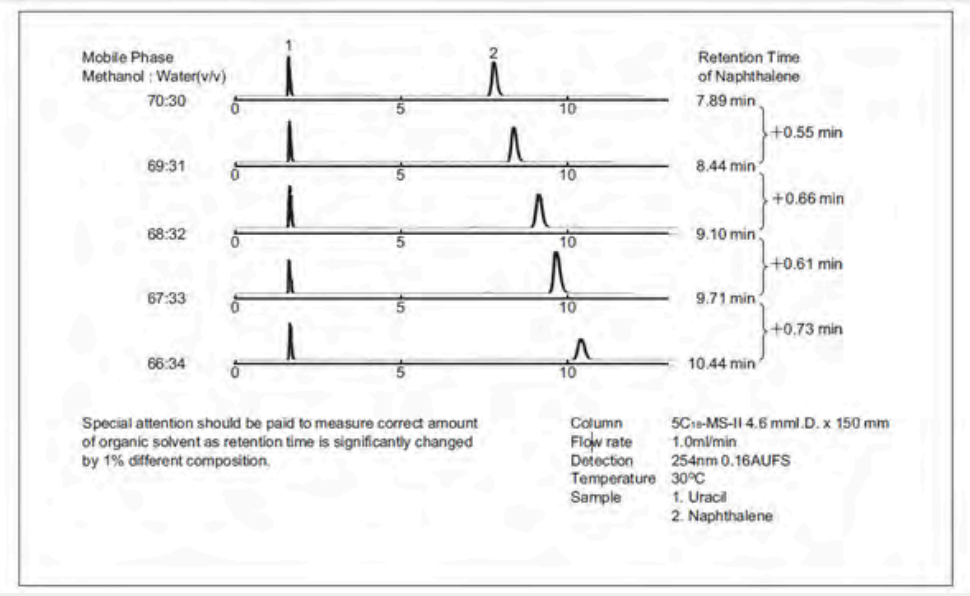
The various effects offered by the mobile phase influence the retention and differential migration (selectivity) of the solutes through the column. Therefore, during method development and optimization, the separation might have to be tuned by changing mobile phase parameters, such as solvent type, additives (different buffers, ion pair reagents), or operating conditions (gradient time/steepness, temperature, flow rate). Often, problems with chromatographic separation are related to an incorrectly/inconsistently prepared mobile phase. Hence, an inclination to use simpler mobile phases can be observed for practical reasons of increased method robustness, easier method transfer, and ease of use.

When selecting the correct purity of mobile phase components, it is an absolute must to use gradient grade solvents & reagents for gradient grade separations to get an accurate, reproducible and clean baseline (free of ghost peaks), and sensitive chromatographic separation. Using the correct and suitable grade of solvents based on the application (e.g. for HPLC) also minimizes the chances of contaminations and extends the longevity of a chromatographic column. For cases requiring addition of any reagent like buffer, it is to be ensured that the reagent meets the required quality and has not passed its expiry date.

The degradants from expired additives lead to ghost peaks in sample chromatograms. Certain additives degrade quicker, depending on their nature (for example 20 mM, pH 7 phosphate buffer). Improper/careless handling (for example left over solvent put back into bottle, bottle left on the lab bench without the cap closed, lost pipette tips floating inside the bottle and so on) of these reagents spoil chemicals quickly.

Influence of organic solvent composition in mobile phase on the retention time

For isocratic separations with premixed mobile phases, solvent volumetric contractions in commonly used mixtures (water/acetonitrile, methanol or tetrahydrofuran) should be taken into account during their preparation. The only correct way to prepare such mobile phase mixtures is to separately take precisely measured volumes of the components and mix them.



For example, to get a 70% organic mobile phase, 300 mL of water and 700 mL of organic solvent should be precisely measured separately and then combined together in a flask. But if only the water is measured precisely and the organic solvent is then added to make up the required final volume, due to the solvent mixture contraction, the resulting solvent strength will be a little higher (or weaker in case organic solvent was added first and water was added later). For premixing of MPs, attention should be taken to the toxic solvent fumes that might be emitted, under a fume hood.

Nowadays, gradients are generally correctly formulated using gradient pumps; however, some minor differences in retention behavior might be observed during comparison of the instruments with low pressure and high-pressure gradient systems due to their mixing mechanisms.

The final step in the mobile phase preparation is **filtration**. There are many different types of filters that could be chosen based on the solvents being filtered. Recommended are membrane-type filters with pore sizes of at least 0.45 µm for HPLC systems, and 0.22 µm for UHPLC systems. Filtering removes particles from the prepared mobile phase and prevents clogging of the system and column.

Decreasing the baseline

In many cases, the type of solvent and additives used and their purity in the eluent are strongly responsible for the noise of the baseline. Additives such as TEA or TFA might increase noise due to their relatively high UV absorbance. It is particularly important if the detection is done at low wavelengths of below 220 nm. First, check whether or not the detection can also be performed reliably at longer wavelengths. Double check the eluent selection, for example, methanol exhibits a higher absorption at low wavelengths, which can make the detection of smaller peaks difficult.

Uncontrolled temperature drifts during the day for detector and the solvents should also be avoided. Next, even minor impurities from the column after synthesis or because of column bleeding can greatly increase baseline noise. Therefore, it is best to first install a new guard column and, if necessary, also a new separation column and compare the obtained chromatograms.

Another factor to be considered is the HPLC system itself. It can be checked for any contamination or air in the system, performance of pumps (pressure fluctuations), lifetime of UV lamp, and cleanliness of the detector cell - all of which can contribute to the baseline noise.

IFinally, the size of solvent mixing unit. A smaller size offers less contribution to the dead volume, but a higher baseline noise, usually because of less perfect mixing. A larger solvent mixing unit would facilitate a better mixing but would also contribute to larger dead volume. In general, regular maintenance, cleaning cycles, and good understanding of the system's individual components are prerequisites for a problem-free HPLC analysis.

Increasing the signal intensity

Decrease the column internal diameter (ID). The ID of the HPLC column affects the concentration of the sample in the column. Samples are diluted in proportion to the cross-sectional area of the column and therefore, smaller ID columns yield less dilution.

Just a decrease by half of the diameter will result in a ~4 times higher concentration in the detector. Keep in mind that the column capacity is also reduced at the same time and hence the injection volume as well as the flow rate must be adjusted. However, above mentioned increase in sensitivity will be obtained even after adjusting/lowering the injection volume.

Increasing column efficiency

Reduction of particle size causes an increase in the sensitivity because of more narrow and higher peaks. These will simulate a smaller diameter (more efficient) particle without a larger increase in backpressure. For example, replacing a fully porous 3 µm particle packed column with a superficially porous particles of 2.7 µm, would almost double the column efficiency. Since the efficiency is higher, the peak will be narrower and higher, and by that the sensitivity will increase.

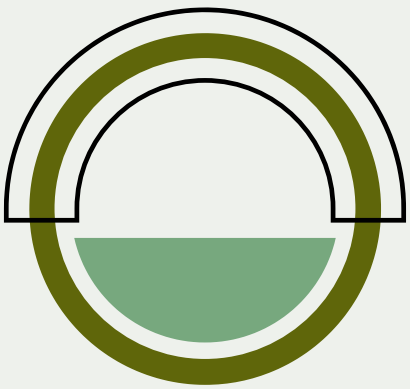
What if you don't get a suitable separation?— or worse, if you don't realize the separation is suitable (i.e. due to analyte co-elution) — without a good underlying we need to be able to realize something has gone wrong and act upon it. The more generic we make things, the less we think about what non-optimal might look like, and the harder it is to correct.

1.You have to recognize when peak shapes are affecting resolution or reproducible integration.
2.You have to recognize when retention time variability, due to small changes in eluent pH when analyzing ionogenic compounds, may be leading to poor resolution in a chromatogram or poor quantitative results (and how to act accordingly).
3.You have to understand the links between sample diluent, injection volume, and pH control (for example, buffering) to avoid peak shape and retention time issues.

4.You have to understand what to do in order to optimize gradient conditions in order to separate analytes whose structure and/or physico-chemical properties are similar, without knowing something of the nature of gradient HPLC and ways of predicting gradient range and slope.
5.You have to develop a sense of when trying to optimize a method is futile and you require a radically different approach—such as a change in stationary phase chemistry, or a change to the mode of chromatography.

For example:
Acetonitrile generally produces high peak capacities, has low UV cut-off, and lower viscosity to help keep back-pressures low. The formic acid at pH 3 will hopefully improve analyte ionization efficiency in electrospray mass spectrometer sources, if MS detection is preferred.

Otherwise, we often revert to, change something in the eluent or method (potion), give it a stir, and see what happens, without any idea of what is producing the change or what to do if it doesn't work.



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HPLC Tips & Tricks - Mobile Phase Preparation Dr. Egidijus Machtejevas, Lead Expert, Analytical Science Liaison, Analytix@merckgroup.com

Liquid chromatography: Tips, tricks & guidance: A guide for improving column selection, sample preparation and method development

Tarun Belwal, Shahira M. Ezzat, Luca Rastrelli, Indra D. Bhatt, Maria Daglia, Alessandra Baldi, Hari Prasad Devkota, Ilkay Erdogan Orhan, Jayanta Kumar Patra, Gitishree Das, C. Anandharamakrishnan, Lourdes Gomez-Gomez, Seyed Fazel Nabavi, Seyed Mohammad Nabavi, Atanas G. Atanasov. A critical analysis of extraction techniques used for botanicals: Trends, priorities, industrial uses and optimization strategies, Trends in Analytical Chemistry 100 (2018) 82e102

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Appendix 4.2
Tricks and tips in GC analysis

Violeta-Alexandra Ion,
Oana-Crina Bujor,
Liliana Bădulescu

Selection of appropriate column

When selecting a column, the analytical chemist's single most essential choice is that of the column stationary phase. The chemical makeup of the stationary phase is what governs the selectivity parameter, determining which compounds are best retained and the order in which they elute. With non-polar phases, the separation is loosely driven by compound boiling point. However, to resolve compounds with similar boiling points (e.g., isomers), highly selective and/or polar stationary phases are often required.

If possible, you should begin by consulting sample applications provided by GC manufacturers and suppliers – or described in published Application Notes. While an exact example application may not be available, enough information can usually be obtained to simplify the decision or reduce the number of potential columns. The most difficult situation is when no previous information is available. Stationary phase selection is much easier even if only one chromatogram is available for all or most of the sample compounds.

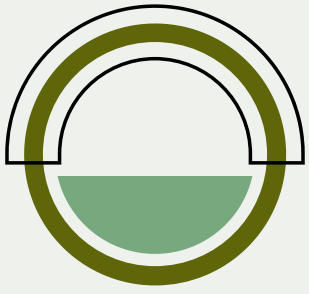
There are four major column parameters to consider: stationary phase, diameter, length, and film thickness.

1. Choose a **stationary phase**– your most critical decision– based on factors such as selectivity, polarity, and phenyl content.

2. Understand how column **diameter** influences factors like efficiency, solute retention, head pressure, and carrier gas flow rates.

3. Determine which column **length** will affect solute retention, column head pressure, column bleed – and cost.

4. Appreciate the difference between **thin-film** and **thick-film** columns with regard to capacity, inertness, bleed, and upper temperature limit. (Agilent Guide 1)



Tabel 4.1.-2. Examples of applications on columns (Restek Guide 1)

Crt. no	Stationary phase	Application
1	50% methyl / 50% phenyl polysiloxane	General-purpose mid-polarity phase, ideal for antioxidants.
2	65% diphenyl / 35% dimethyl polysiloxane	Application-specific column, designed for triglycerides
3	80% dimethyl / 20% diphenyl polysiloxane	General-purpose low to mid-polarity phase, ideal for flavor compounds, alcoholic beverage analysis.
4	50% cyanopropylmethyl / 50% phenylmethyl polysiloxane	General-purpose polar phase, ideal for FAMEs, carbohydrates, sterols, flavor compounds.
5	Carbowax® polyethylene glycol	General-purpose polar phase, ideal for FAMEs, flavor compounds.
6	Trifluoropropylmethyl polysiloxane	General-purpose mid-polarity phase, ideal for alcohols, ketones, glycols.

Selection of appropriate inlet liner – Restek 2

There are different types injection used in GC: split, splitless, direct, gas, and PTV (Programmable Temperature Vaporization). The inlet's main purpose is to transfer the sample onto the GC column for analysis. Choosing the correct GC inlet liner is critical in assuring that the desired amount of sample is transferred onto the column in an efficient manner, without negatively impacting the target compounds.

Two main factors should be taken in consideration when selecting the inlet liner:

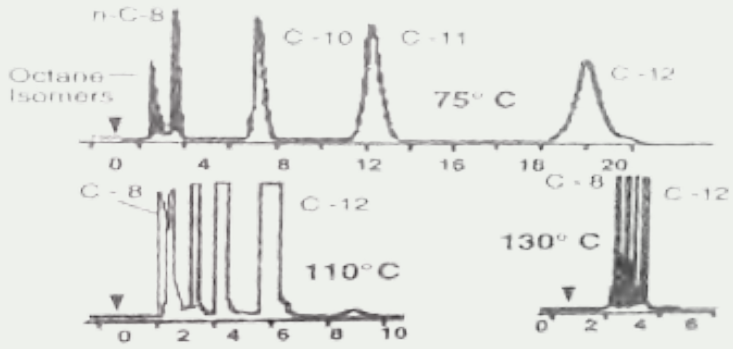
1. Sample concentration

- Use a split injection when compounds of interest are present in high concentration, or when you don't need low limits of detection. In a split injection, only the desired amount of sample is transferred onto the GC column—avoiding column overload and prolonging column life.
- Choose a spitless injection when compounds of interest are present at low concentration levels. This technique involves closing the split vent at the start of the injection, directing all the flow passing through the inlet to the column. At the end of a set period (the purge time), the split vent is opened to flush out any remaining vaporized solvent.
- Direct injection is best when compounds of interest are at trace levels, and contact between the sample and inlet seal (or wool) could cause degradation or adsorption. With direct injection, the sample is injected into a hot inlet, vaporizing the entire sample into the GC column

2.GC solvent vapor volume

The volume of sample introduced into a heated liner increases greatly during vaporization. How much it expands is determined by the solvent, the inlet temperature, and the pressure inside the liner.

Your liner volume needs to be large enough to accommodate the gaseous sample. If the diameter is too small, the sample will expand beyond the liner's capacity—causing sample loss through the septum purge flow and split line. When the sample doesn't transfer to the column, it can lead to peak tailing, poor peak area reproducibility, and carryover.



Effect of temperature on retention time
(Souce: Harold M. McNair & James M. Miller, Basic Gas Chromatography, 1998, John Wiley & Sons, New York.)

Your liner volume needs to be large enough to accommodate the gaseous sample. If the diameter is too small, the sample will expand beyond the liner's capacity—causing sample loss through the septum purge flow and split line. When the sample doesn't transfer to the column, it can lead to peak tailing, poor peak area reproducibility, and carryover.

Bonus: When selecting an optimal septa are those that are low-bleed, have a temperature tolerance of 400 °C, can withstand 100 – 150 injections before becoming cored, and feature a hole to facilitate needle entry.

Selection of appropriate mobile phase (gas)

In GC there are three gases that are commonly used as a carrier gas: nitrogen, helium and hydrogen. The importance of carrier gas selection has been a discussion point amongst the users of GC for many years. When selecting the right carrier gas the user has to consider different parameters such as: performance, analytical compatibility or just availability.

It is advisable to use the highest purity gas possible. Ultra high purity (99.99%), ultrapure carrier (99.995%), or even research grade (99.9999%) is preferred to minimize critical impurities, instrument downtime and troubleshooting. Air, moisture and organic traps should be used, but it is better to start with the highest purity gas and reduce the load on gas purifiers as much as possible.

Helium should be used for capillary GC whenever possible; nitrogen shows inferior performance due to slow optimum linear velocity.

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Appendix 4.3
Tricks and tips in ICP-MS analysis

Carmen Constantin,
Aurora Dobrin,
Liliana Bădulescu

In ICP-MS analysis, there are a number of factors that can critically impact the quality of the data such as: the purity of the reagents and the sample preparation method.

If possible, you should begin by consulting sample applications provided by GC manufacturers and suppliers – or described in published Application Notes. While an exact example application may not be available, enough information can usually be obtained to simplify the decision or reduce the number of potential columns. The most difficult situation is when no previous information is available. Stationary phase selection is much easier even if only one chromatogram is available for all or most of the sample compounds.

Blank solution

The analytical blank used in ICP-MS analysis must be without any trace of analytes.
This blank should be treated as a sample and all of the steps of the analytical procedure should be applied in order to obtain a blank solution.

Each of these factors is related to the reduction and to the control of the analytical blank.

- the acid concentration should be lower than 10% V/V and the best acid medium for sample preparation is nitric acid because when we introduce this acid into the argon plasma, we are introducing elements (i.e. H, N, and O) already present in the plasma due to air diffusion or to water dissociation.
- when working with more viscous acids, such as sulfuric or phosphoric acid, it is better to keep the maximum concentration around 5%

Internal standard

When using an internal standard, the analyte concentration is related to the ratio between the analyte signal and the internal standard signal. This strategy allows the correction of certain interferences and it is particularly effective to correct transport interferences during sample introduction by pneumatic nebulization.

Loose in sensitivity

Can be given by: a blocked nebulizer, partially blocked injector in torch (rare), partially blocked interface cones, poor optimization, extract/Omega lens needs cleaning, insufficient argon pressure or flow, tuning – use of non-standard tune settings and contaminants in reaction gas (O2, H2O, organic etc.).

Precision in analysis

Can be affected by: old calibration standards (contamination, precipitation, evaporation, etc.), preparation of the standards, differences in stock solutions between suppliers, insufficient stabilization time, incomplete digestion (particles in solution), wash-out (memory effects).

ICP-MS Sample Introduction System Tips

- Make MassHunter performance report at beginning of day;
- Check/adjust the peri pump tubing;
- Check the blank reading;
- Rinse before samples and at the end of the analysis;
- Clean the cones/extract lens as needed;
- The cones must be prepared after cleaning if running high matrix samples;
- DON`T use DI water as rinse water;
- DON`T wait until the last minute before maintaining the system (pump oil, peripump tubing, cone cleaning, lens cleaning etc.).

Peri Pump Tubing Tips

- Tubing used for waste must be larger than sample tube;
- Ensure tubing is resistant to the solvent being used;
- Precondition new tubing before analysis (wash, stretch);
- Using old tubing may affect the precision and stability;
- The lifetime is about 5 days based on normal 8 hour working day;
- When the analysis is complete, detach from tube holder– allows tube to “relax”.

Signal pulsation Tips

- Check the shape of the tubing– should not be any flat spots;
- Peri pump tubing should be elastic – replace if obviously stretched;
- Don’t over tighten – just need a smooth sample flow;
- Peri pump tubing that looks/feel worn or has a strange color-change it;
- Irregular sample flow-check peripump clamp tension;
- If there are bubbles in the liquid stream-check sample introduction fittings, tubing and connectors for loose connections.

Sample Introduction–Nebulizer Tips

- Rinse at least 10 minutes with a reagent blank before extinguishing plasma.

Tips to Reduce Contamination

- Contamination can occur from anything that comes into contact with the sample during storage, digestion, dilution and analysis;
- Always use the best reagents necessary for your analysis – use high purity or trace metal grade reagents;
- Reseal immediately after use.

Other common contamination sources can be: reagent water, borosilicate glass can contribute Boron contamination and airborne dust in the lab

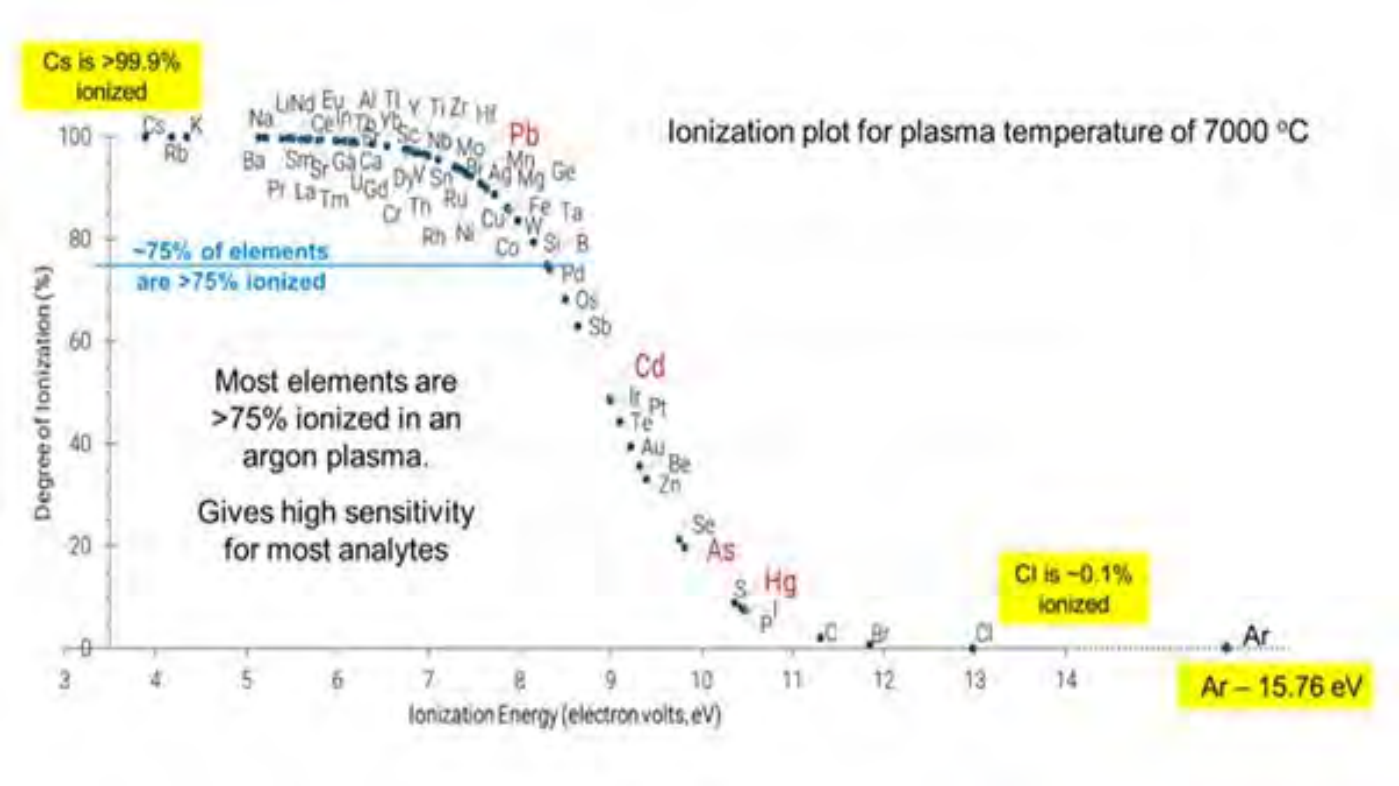
Pipette tips

- Don’t insert pipette tips into your acids bottles;
- Don`t use colored tips-this may increase contamination (Cu, Fe, Zn, Cd);
- DON`T use powdered gloves (esp. for Zn);
- ICP-MS –Autosampler Issues to consider:
- Longer transfer tube between sampler and ICP-MS –you need to set a longer sample uptake time;
- Dust can introduce contamination;
- Sample evaporation may occur during long storage;
- Sediment in the sample may settle out;
- The sample transfer line to ICP-MS must be clean.

The equipment's stability may be checked by measuring one reference solution during different operation times and comparing the obtained signals.

It is also highly recommended to use certified reference materials to evaluate the accuracies of all the determined elements.

TR- just revise



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The Principles of ICP MS
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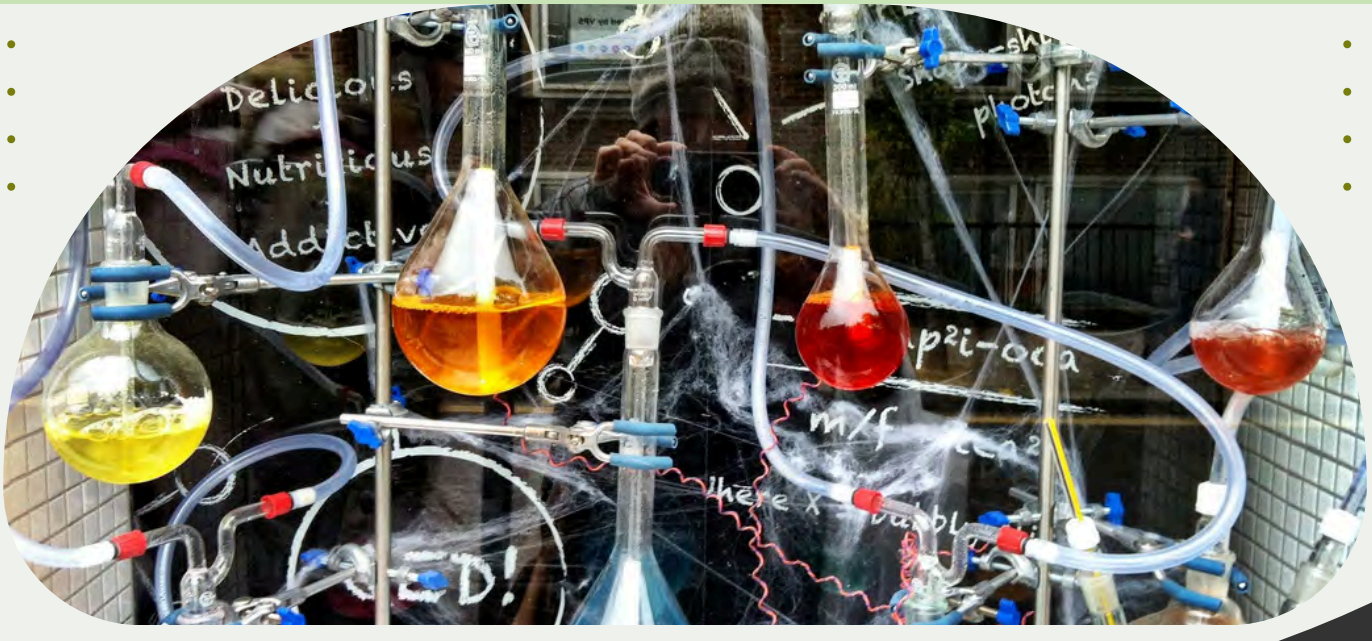
University
of Agronomic Sciences
and Veterinary Medicine
of Bucharest

021 318 2266
https://hortgreen.com/
Address: Bulevardul Mărăști 59, București 011464



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