WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 10, 1145-1178. Review Article ISSN 2277– 7105

ANALYSIS OF NUTRACEUTICALS BY CHROMATOGRAPHY

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Article Received on
02 May 2023,

Revised on 22 May 2023,
Accepted on 12 June 2023

DOI: 10.20959/wjpr202310-28379

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ABSTRACT

Nutraceuticals are foods that benefit human health in a therapeutic way. It consists of herbal remedies, probiotics, prebiotics, and medical foods designed for illness prevention and therapy. Because of their alleged safety, nutraceuticals have attracted a lot of interest. These dietary supplements aid in the fight against some of the biggest health issues of the twenty-first century, including diabetes, osteoporosis, cancer, cardiovascular illnesses and cholesterin etc. Because they don't produce side effects, have naturally occurring dietary supplements, etc., nutraceuticals offer an edge over medications. Nutritional supplements, herbal remedies, and dietary supplements, among others, are categorised as nutraceuticals based on their natural source and chemical grouping. Dietary supplements and natural/herbal goods saw the industry's fastest growth rates. Analytical methods used to detect and/or quantify the many nutraceuticals found in natural matrices with a focus on more modern methods. The work is distributed according to the different methods available for the analysis for the nutraceuticals (separation, spectroscopic, hyphenated techniques, etc.). Information about the claimed health promoting effects of the different families of nutraceuticals and Techniques are also provided together with data on the natural matrices in which they can be found (e.g., fruits, vegetables, plants, microalgae, cereals, milk, etc.).

INTRODUCTION

Nutraceuticals is a term derived from “nutrition” and “pharmaceutics.” Products that are separated from herbal items, dietary supplements (nutrients), certain diets, and In addition to providing nourishment, processed meals like cereals, soups, and beverages also serve as medicines. Products known as nutraceuticals can be used as medication in addition to being nutritional. One definition of a nutraceutical product is a chemical that benefits physiological function or offers defence against chronic disease.[1]

Nutraceuticals can be used to boost wellbeing, slow down ageing, prevent chronic diseases, lengthen life expectancy, or support the body's structure or functions. Due to their potential for having nutritional, safe, and therapeutic impacts, nutraceuticals have recently attracted a lot of attention.[2]

These medicines have demonstrated promising outcomes in a variety of problems, according to recent investigations. Much work has gone into the current review to propose novel ideas regarding nutraceuticals based on their potential to treat or prevent diseases. The presentation of herbal nutraceuticals that are helpful against difficult-to-treat oxidative stress-related diseases including obesity and diseases like Alzheimer's, diabetes, cancer, Alzheimer's, cardiovascular, inflammatory, ocular, and immunological disorders has received particular attention.[3]

Nutraceutical goods are classified as medications, food additives, and dietary supplements in the US. Contrary to medications, nutritional supplements typically lack patent protection. Although both pharmaceutical and nutraceutical substances may be used to treat or prevent disease, only pharmaceutical substances are approved by the government. A product is deemed to be a dietary supplement if it bears or contains one or more of the dietary components listed below: a concentration, metabolite, constituent, extract, or a combination of any one or more of the following: a mineral, vitamin, amino acid, medicinal plant or other botanical, nutritional supplement used by humans to boost daily caloric intake.[4]

Nutraceutical use is growing fast and is well accepted by people for its all-natural origin. Nutraceuticals cannot replace pharmaceuticals but can be used in the prevention and cure of some pathological conditions. According to Stephen DeFelice (1989), a nutraceutical is a food or part of food capable of providing beneficial health effects, including the prevention and the treatment of disease, which is not applicable to food supplements. Nutraceuticals can

be found in meals with either plant- or animal-based origins, and there has been much research on their mechanism of action, safety, and clinical outcomes. These therapeutic agents don't advertise themselves as alternatives to medications; instead, they can be useful in preventing a group of illnesses known as the metabolic syndrome, such as type 2 diabetes, stroke, heart disease, and cardiovascular disease.

The future of prevention and therapy is a challenge for nutraceuticals, which are also a catalyst for medical research. A potent instrument to combat pathological, chronic, and long-term diseases in subjects who do not qualify for pharmacological therapy is the potential for preventing or supporting pharmacological therapy, which is today mostly based on pharmaceuticals.[5][6]

Classification of Nutraceuticals

1. NON-TRADITIONAL NUTRACEUTICALS[7]

These Are artificial foods prepared with the help of biotechnology. Food samples contain bioactive components which are engineered to produce products for human- wellness.

They are arranged into two types.

A. Fortified nutraceuticals.

B. Recombinant nutraceuticals.

A. Fortified nutraceuticals

They are often enhanced with vitamins and minerals up to 100% of the Dietary Reference Intake for each nutrient. Nutraceuticals with agricultural breeding or additional nutrients are known as fortified foods. Orange juice fortified with calcium, cereals fortified with vitamins or minerals, flour fortified with folic acid, and milk fortified with cholecalciferol are a few examples of fortified nutraceuticals.

B. Recombinant nutraceuticals

Production of probiotics and obtaining bioactive ingredients components produced using enzyme and fermentation technologies because genetic engineering technology is made possible biotechnology. foods that provide energy, like bread vinegar, yoghurt, cheese, fermented starch, and Others are created with biotechnology's assistance.

2. TRADITIONAL NUTRACEUTICALS[8][9]

Traditional nutraceuticals are simply natural with no changes to the food. Food contains several natural components that deliver benefits beyond basic nutrition, such as lycopene in tomatoes, omega-3 fatty acid in salmon or saponins in soy.

A. probiotic micro-organism

B. chemical constituents

C. nutraceutical enzymes

A. Probiotic Micro-organisms

They work to eliminate pathogens like yeasts, other bacteria, and viruses that may otherwise cause illness and form a beneficial relationship with the gastrointestinal system of a human. Through altering the microflora, preventing pathogen adhesion to the intestinal epithelium, competing for nutrients required for pathogen survival, producing an antitoxin effect, and reversing some of the effects of infection on the intestinal epithelium, such as secretory changes and neutrophil migration, they have an antimicrobial effect. By producing the exact enzyme (ß-galactosidase) that can hydrolyze the problematic lactose into its component sugars, probiotics can treat lactose intolerance. As an illustration, yoghurt is one of the best sources of probiotics, which are beneficial microorganisms.

B. chemical constituents

HERBAL

With the aid of herbs, nutraceuticals show considerable promise for enhancing health and preventing chronic disease. Aloe Vera gel is one illustration. Capillary dilation, anti-inflammatory, moisturising, and wound-healing qualities. Ephedra is a vasoconstrictor and bronchodilator that also lessens bronchial edoema. Garlic: Thrombotic, antibacterial, antifungal, hypotensive, and anti-inflammatory. Liquorice: a peptic ulcer therapy, expectorant, and secretolytic. Ginger is a cholagogue, antiemetic, carminative, and positive inotropic.

PHYTOCHEMICALS

Phytochemicals are essentially plant nutrients with unique biological properties that enhance human health and fend off numerous damaging human diseases. A few instances Many fruits, vegetables, and egg yolks include carotenoids (isoprenoids), which have anti-carcinogenic properties, are also known to stimulate natural killer immune cells and shield the cornea from UV rays. Non-carotenoids, which lower cholesterol and fight cancer, are found in grains,

legumes (such as chickpeas and soybeans), and palm oil. The immune system can be strengthened by sulphides, which are present in onions and garlic. Foods high in phytochemicals include tomatoes, apples, apricots, broccoli, Brussels sprouts, cabbage, carrots, cauliflower, garlic, legumes, onions, red peppers, and sweet potatoes.

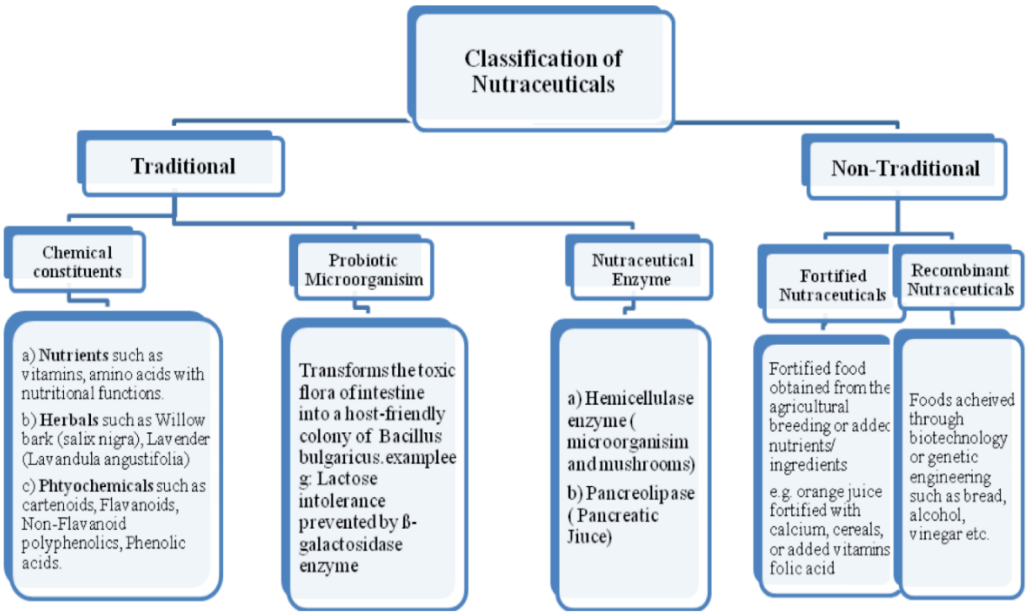


Fig 1: Classification of Nutraceuticals.

C. nutraceutical enzymes

Without enzymes, life would not be possible and our bodies would stop working. Adding enzyme supplements to one's diet can help patients with medical diseases like hypoglycemia, blood sugar imbalances, digestive issues, and obesity get rid of their symptoms. These enzymes come from animal, plant, and microbial origins. Examples include the enzyme Xylanase, which comes from Trichoderma sp. Benefits: Xylanase breaks down high molecular weight arabinoxylans and can be used to treat vegetable proteins and feed grain endosperm cell walls. The inclusion of Xylanase to feed provides remedies for a number of issues related to arabinoxylans. As a protease enzyme to aid in protein digestion, papain enzyme is widely employed in the nutraceuticals business.

Need of Analysis of the nutraceuticals

Unstandardized approach to nutraceutical regulation puts a large responsibility on supplement manufacturers to ensure a high level of quality controls to avoid endangering consumers, not to mention hefty fees and penalties. While specific regulations may vary by country, manufacturers should always take responsibility for traceability and quality control

throughout the entire procedure. Many, if not all, risks can be mitigated with thorough testing at each step, including but not limited to.

Ingredients – Testing must begin with pathogen detection (and identification), and quality indicators enumeration for raw materials and ingredients.

Formula – Perhaps the most important step of the production process is the supplement formula, not only the list of finalized ingredients but a functional, repeatable measurement of the level present for each.

End-products Testing (Required & Non-Required) – In cases where rigorous testing is not required, manufacturers are responsible for purity, efficacy, and allergen testing. Manufacturers may choose to obtain a USP Verification Mark, proving that their supplements abide by their label, do not contain harmful levels of contaminants, will be digested in a specified amount of time, and have met by GMP standards.

An additional problem related to the production and consume of nutraceuticals is that the composition and contents of active constituents in natural plants (like in any other natural source) vary depending on season, climate, temperature, humidity, soil and several other factors. So the collection, identification and maintenance of uniform quality, quantification and standardization are critical factors to consider.

The other imp points due to which the analysis of nutraceuticals are required to be done are as below.

- To identify the authentic source of raw materials.
- To identify the purity of the compound.
- To identify the presence of other active compounds.
- To identify the quality.
- To avoid the false advertising.
- To identify the contamination with heavy metals.
- To identify the interactions between supplements and drugs.

Analysis of Nutraceuticals[10][11][12]

- Analysis of Nutraceuticals includes the identification of new nutraceuticals, characterization of their chemical structure and bioactivity, quantification in the natural source, product development, quality control in their dosage forms, etc.
- Several no of techniques are available for the analysis of the nutraceuticals and the development of advanced analytical techniques is, therefore, indispensable in nutraceuticals research.
- Due to the complexity of the natural matrices, the use of advanced analytical techniques (such as Mass Spectrometry (MS), Nuclear Magnetic Resonance (NMR), High performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE), HPLC-NMR, HPLC-MS, GC-MS and CE-MS) is mandatory in order to carry out the studies.
- Some of these methods are already used to confirm the composition of natural products from lot to lot and to guarantee the end product's safety. Additionally, at the early stages of their discovery, these methodologies are frequently coupled with each other for product development, primarily to address the difficulty of analysing several components or various classes of components.
- The choice of the analytical technique depends also on the target compounds and the matrix in which they can be found.
- Additionally, sophisticated analytical methods are required to learn more about the health-promoting properties of nutraceuticals and to determine how well these chemicals are absorbed by the body. Important aspects during product development should include nutraceuticals bioactivity and bioavailability studies, so, in vitro, in vivo and clinical trials should ideally be employed.
- Nevertheless, many nations' present legislation on these chemicals is less stringent than that for regular medications, which typically leads to little trials to validate their activity.
- For example, their physico-chemical properties (polarity, size, volatility) will have a strong influence onto the sample preparation procedure, separation mechanism and technique (GC, HPLC, CE) and the type of detector to be employed (Ultraviolet Detector (UV), Fluorescence Detector (FLD), Flame Ionization Detector (FID), MS, etc.).

Analysis of Nutraceuticals using Different Chromatographic Techniques

HPLC (High-performance liquid chromatography)[13]

High-performance liquid chromatography is known as HPLC. Chromatography refers to the method of measurement, chromatogram to the output of the measurement, and

chromatograph to the apparatus. Chromatography separates the constituent parts of a substance and then analyses the constituent parts on a qualitative and quantitative level. Quantitative analysis refers to "how much of each component is there," whereas qualitative analysis relates to "what kind of compound each component is."

Separation mechanism

Moving a mixture through a stationary phase, which is a solid medium, involves placing it in a liquid stream known as the mobile phase. The mixture's constituents interact with the stationary phase and move with the mobile phase's flow. The intensity of the interactions between each component and the stationary phase determines how quickly things move. In other words, components that have strong interactions with the stationary phase move slowly, whereas components that have weak interactions move quickly, allowing the components to be separated.

HPLC system configuration

Column chromatography, a type of separation technique known as high-performance liquid chromatography (HPLC), was created to permit quick separation and analysis using high pressure. Five components make up an HPLC system: a pump for delivering liquid, an injector for introducing samples, a column for separating substances, a detector, and a data processor. The solvent (mobile phase) and sample are delivered to the detector via a pump. The sample is injected into the mobile phase using an injector (manual) or an autosampler (automatic). An oven could be used to maintain a constant temperature in the column for a more stable separation. Depending on the detector type, different separated components are detected by the detector. The data processor displays the detected signal (chromatogram) on a computer and analyses it. The data processor allows identification and quantification of components.

HPLC pumps

pumps are classified according to their flow rate.

- Nano LC pumps: 1 µL/min or less
- Micro LC pumps: several tens of µL/min
- Semi-micro LC pumps: several hundreds of µL/min
- Analytical pumps: several mL/min
- Preparative pumps: several tens of mL/min or more

The pump flow rate for normal analysis is several mL/min.

Types of detector

Depending on the target sample, different detectors might be employed. The detector you choose will rely on the samples and needs of your application. The most popular detectors for general use, spanning a variety of components and applications, are UV or PDA detectors. A mass spectrometer or a fluorescence detector can be utilised when more sensitivity is needed. It is better to use an evaporative light-scattering detector or a differential refractive-index detector for a more general detection for substances that don't absorb or fluoresce.

Normal phase vs. reversed-phase

Reversed-phase chromatography is a totally distinct technique from normal-phase chromatography. The low-polarity components are eluted first in normal-phase chromatography, which involves passing a low-polarity solvent through a high-polarity column. The most frequently used approach, reverse-phase chromatography, involves passing a more polar solvent through a non-polar (typically C18 or similar) column with polar components eluting first.

Quantitative analysis using HPLC

There are two quantification techniques: the internal standard method and the external standard method, both of which rely on a calibration curve. The unknown samples are quantified using the calibration curve that was previously established for the reference sample using the external standard method. When producing a calibration curve with a standard sample using the internal standard method, a predetermined amount of an internal standard substance is introduced to an unknown sample, and a calibration curve is then generated using the concentration ratio vs. peak area ratio for quantification. The internal standard substance must be a component that is absent from the sample itself, produce peaks that are entirely distinct from those of any contaminants, elute at a retention time that is close to that of the quantitative target component, be chemically and physically stable, and be extremely pure. The benefit of using the internal standard approach is that it eliminates errors in injection volume and solvent evaporation-related inaccuracies.

Hyphenated Techniques[14]

Hyphenated techniques combine chromatographic and spectral methods to exploit the advantages of both. Chromatography produces pure or nearly pure fractions of chemical

components in a mixture. Spectroscopy produces selective information for identification using standards or library spectra.

Hirschfeld used the phrase "hyphenation" a few decades ago to describe the fusion of a separation technique and one or more spectroscopic detection techniques. This method, which was created by combining a separation method and a spectroscopic detection method, is now referred to as a hyphenated method.

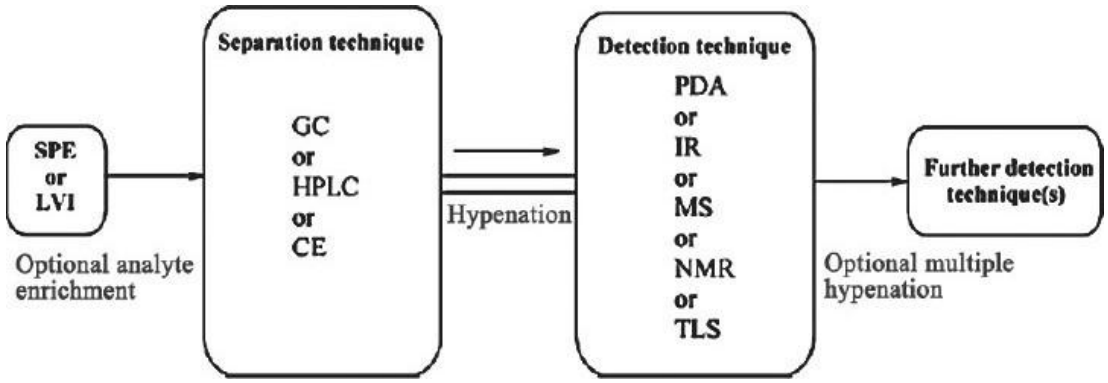


Fig 2: Hyphenated Techniques.

Hyphenated approaches have drawn more and more attention in recent years as the primary method for resolving challenging analytical issues. In order to analyse unknown substances in complicated natural product extracts or fractions, both quantitatively and qualitatively, it is powerful to combine separation methods with spectroscopic techniques.

Liquid chromatography (LC), typically high-performance liquid chromatography (HPLC), gas chromatography (GC), or capillary electrophoresis (CE) are connected to spectroscopic detection techniques to gain structural information leading to the identification of the compounds present in a crude sample, e.g., Fourier-transform infrared (FTIR), photodiode array (PDA) UV–vis absorbance or fluorescence emission, mass spectroscopy (MS), and nuclear magnetic resonance spectroscopy (NMR), resulting in the introduction of various modern hyphenated techniques, e.g., CE-MS, GC-MS, LC-MS, and LC-NMR. HPLC is the most widely used analytical separation technique for the qualitative and quantitative determination of compounds in natural product extracts.

The ability to solve structural issues of complicated natural products has expanded because to the physical connection between HPLC and MS or NMR. LC-MS has been utilised more often than LC-NMR because to its higher sensitivity. The coupling of separation and

detection techniques might involve more than one separation or detection approach, therefore the hyphenation does not always have to be between two techniques, e.g., LC-PDA-MS, LC-MS-MS, LC-NMR-MS, LCPDA-NMR-MS, and the like.

Hyphenated HPLC Methods Used for Analysis of Nutraceuticals

1. HPLC-UV[15]

A chromatography system is, at its core, relatively straightforward: it consists of a way to introduce the sample, stationary and mobile phases to separate molecules, and a way to identify the molecules that have been separated. Chromatography's strength as an analytical technique is its capacity for post-separation analysis on a sample.

Flame-ionization, thermal conductivity, and electron capture are only a few of the frequently employed detectors in chromatography, which uses a wide variety of detectors. Ultra violet absorption, however, may be the detector utilised in liquid chromatography the most frequently, giving rise to the method HPLC-UV.

A UV detector makes use of the capacity of molecules to absorb ultraviolet light; detectors can employ a single wavelength or a range of wavelengths that can be more sensitive. You may identify the specific amounts of each component in an eluting sample mix by shining UV light through the mixture and measuring how much of it is absorbed by each component.

Fig 3: HPLC-UV Instrument.

The Nutraceuticals analysed with HPLC-UV are given in the following Table 1.

Nutraceuticals	Matrix	Health Effect	Ref. No.
Terpenoids	Quinoa flour (pseudo-cereal)	Antibacterial and antineoplastic properties.	[16]
B-carotene, lycopene	Thai fruits	Antioxidant, anti-cancer, prevent degenerative diseases	[17]
Vitamin C (L-ascorbic acid)	Fruits	Antioxidant	[18]
L-ascorbic acid dehydroascorbic acid)	Buckwheats	Antioxidant	[19]
S-methyl-L-methionine (vitamin U)	Centella asiatica	Wound healing	[20]
Type II collagen	Chick	Can suppress Rheumatoid arthritis (RA) and promote healthy joints.	[21]
Saccharides	Black currant pomace	Antioxidant properties	[22]
Glycosides (glucosinolates, glycyrrhetic acid, glycyrrzhin, liquiritin, steroidal glycosides...)	Plants	Choleretic, anti-inflammatory, anti-cancer, antioxidant, anorexant and diuretic properties	[23]
Resveratrol	Nutritional suplements	Antioxidant	[24]
Silymarin	Milk thistle	Antioxidant	[25]
Phytoestrogens	Dietary supplements	Estrogenic activity	[26]
Flavonol glycosides	Ginkgo biloba	Memory enhancing	[27]
Isoflavones	Soybean seeds	Antimenopausial sympthoms	[28]
Acids (bitter acids, asiatic acid and asiaticoside)	Plants (Centella asiatica, hop)	Anticarcinogenic properties	[29]

2. HPLC-MS[30]

High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) is an ideal technique for the identification, quantification, and mass analysis of components. High sensitivity and reproducibility make HPLC-MS the ideal tool for performing accurate quantitative analyses. Techniques for high performance liquid chromatography are excellent for heat-labile chemicals found in food products, such as proteins and vitamins. It is frequently used to ascertain the chemical composition and purity of compounds.

In HPLC-MS, an interface transports the separated components from the liquid chromatograph column into the mass spectrometer ion source, joining the two procedures (HPLC and MS). Since the MS system has a high vacuum and HPLC operates at a high pressure, an interface is required.

Both the mobile phase and the stationary phase in HPLC are adaptable to the sample matrix and the desired qualities to be identified. Typically, the stationary phase is tuned to complement the mobile phase and the mobile phase is modified to fit the sample. Based on the compound's affinity for the mobile phase, the degree of compound separation is determined.

Depending on the characteristics of the stationary and mobile phases, HPLC techniques can be categorised into two broad groups. "Normal-phase chromatography" is the term for the combination of polar stationary phase and non-polar mobile phase, while "reverse-phase chromatography" is the term for the combination of non-polar stationary phase and polar mobile phase.

HPLC-MS is suitable for samples in liquid form. For solid sample matrices, sample preparation is necessary. Compounds that are being determined must be extracted into a solvent and filtered before analysis.

Mass spectrometry (MS), which generates a mass spectrum that is distinct for every constituent, is used to identify compounds after they have been separated using HPLC and to discover what is inside of them. The chemicals and their fragments are ionised in mass spectrometry using either chemical or electron ionisation. The ions are then identified using their mass-to-charge (m/z) ratios after the sample has been accelerated through a mass analyzer that either has a quadrupole or an ion trap.

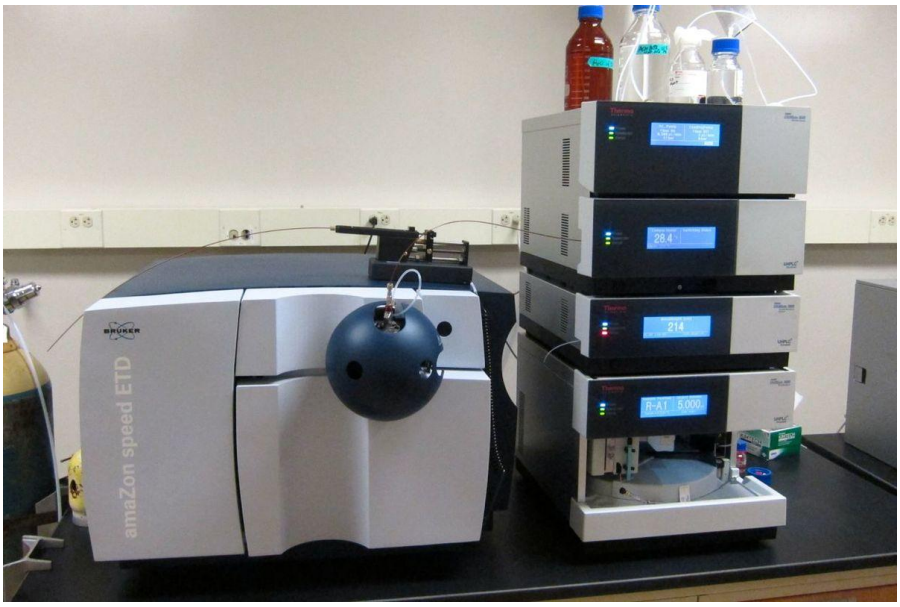


Fig 4: HPLC-MS Instrument[31]

The Nutraceuticals analysed with HPLC-MS are given in the following table 2.

Nutraceuticals	Matrix	Health Effect	Ref. No.
Milk lipids (triglycerides, diacylglycerides, saturated fatty acids and PUFAs).	Milk	Immuno-suppressive, anti-inflammatory, andantimicrobial properties	[32]
Gangliosides	Dairy products (milk)	Protect against enteric pathogens, and prebiotic functions.	[33]
Water-soluble vitamins (B1, B2, two B3 vitamers, B5, five B6 vitamers, B8, B9, B12 and C).	Maize flour, green and golden kiwi and tomato pulp.	Antioxidant and co-enzymes	[34]
Milk proteins, peptides Lactoferrin and immunoglobulin G.	Milk and derived products	Antihypertensive, antimicrobial, anti-inflammatory and inmunostimulating activities. Important source of amino acids	[35]
Lysozyme-derivedpeptides	Hen's egg	Antimicrobial activity	[36]
Cyclopeptides	Cow cockle seed	Estrogen like activity in vivo	[37]
Phenolics	Fruits, Mushrooms, legumes	Antioxidant	[38]
Anthocyanins	Fruits, tubers	Antioxidant	[39]
Flavonoid aglycones	black currant	Antioxidant	[40]
Flavonoids	Cranberry	Antioxidant	[41]
Alkil phenols	Anacardum	Antioxidant	[42]
Monacolins	Rice	Cholesterol lowering and anticancer agent	[43]

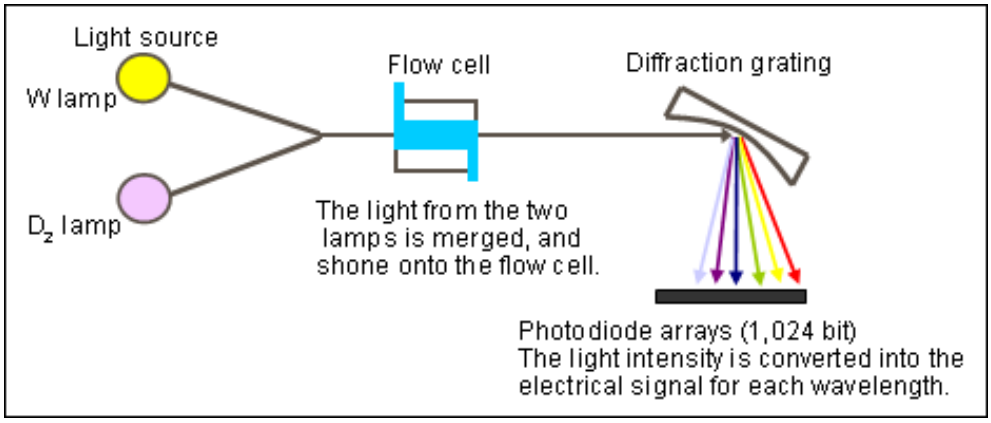
3.HPLC-DAD[44]

In contrast to UV-VIS detectors, DADs estimate the amount of scattered light for each wavelength in the photodiode arrays. Light from the lamps is shone directly onto the flow cell, light that passes through the flow cell is dispersed by the diffraction grating, and light that passes through the flow cell is not detected by the flow cell.

The DAD is inferior to a UV-VIS detector in the following ways: The DAD is vulnerable to numerous changes, such as bulb variations because the reference light cannot be received, and noise is high because there is little light. The DAD has, however, lately undergone improvements to lessen the performance gap between it and UV-VIS detectors.

The concept is that during separation by HPLC with continuous eluate supply, spectra are monitored at intervals of 1 second or less. A little difference in retention time can make it challenging to identify components when the measurement is done at a fixed wavelength because the components can only be identified from their retention duration. In this situation, the DAD can be applied to compare the spectrum and identify components.

The detection unit makes use of semiconductor devices called photodiode arrays. A DAD measures absorption in the UV to VIS range. One advantage of a DAD is that it has several (1024 for L-2455/2455U) photodiode arrays, in contrast to a UV-VIS detector's single sample-side light-receiving portion. This allows a DAD to collect data over a wider wavelength range at once.



The results of a measurement with a DAD are shown in the contour map as in Figure Convenient functions are provided, including a peak purity check and library search, as well as quantitative analysis with a specified chromatogram.

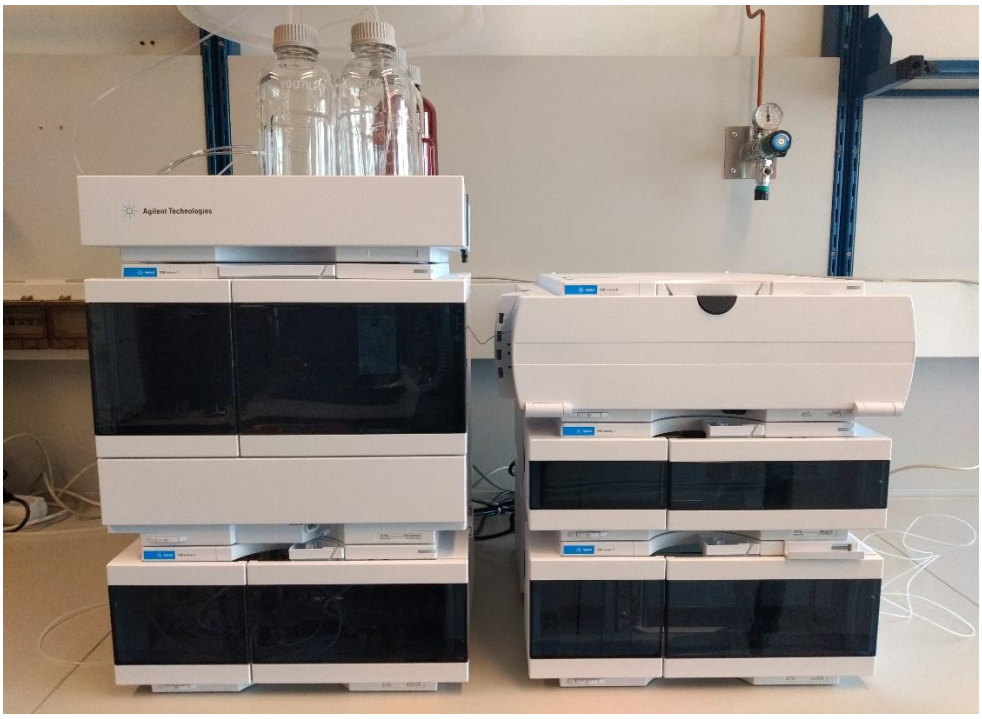


Fig 5: HPLC-DAD Instrument[45]

The Nutraceuticals analysed with HPLC-DAD are given in the following table 3.

Nutraceuticals	Matrix	Health effect	Ref. No.
β-carotene	Tea seed oils	Antioxidant effects	[46]
Astaxanthin, β-carotene, lutein, cantaxanthin, violaxanthin, neoxanthin	Alga	Antioxidant, inmunomodulation and cancer prevention.	[47]
Tocopherols (Vitamin E)	Microalga	Antioxidant and prevents degenerative disorders	[48]
Vitamin B1 and B2	Mushrooms	Antioxidant	[49]
Fat and water soluble vitamins	Beer and bioactive drinks	Antioxidant and co-enzymes	[50]
Peptide	Fishes	Antihypertensive, antioxidant and anticoagulant activities	[51]
Phenolics	Fruits, Mushrooms, legumes	Antioxidant	[52]
Anthocyanins	Fruits, Nutraceutical Capsules	Antioxidant	[53]
Catecholamines	Banana peel	Antioxidant	[54]
Rutin	Buckwheats	Antioxidant	[55]
Flavone isomers	lemon juice	Antioxidant	[56]
Phenolics	Potatoe	Antioxidant	[57]
Phenolic acids	Cooked meat	Antioxidant	[58]
Flavonol	Bean	Antioxidant	[59]
Phenolics	Moscatel sweet wines	Antioxidant	[60]
Flavonoids	Ulmus davidiana	Antioxidant	[61]
Resveratrol	Grape canes	Antioxidant	[62]
Phenolic acids and flavonoids	Glycin tomentella Hayata (Leguminosae family)	Antioxidant	[63]
Isoflavones	Red clover	Antifungal activity	[64]
Isoflavones	Nutritional supplements	Estrogenic activity	[65]
Isoflavones	Soy supplements	Antimenopausial sympthoms	[66]
Phenolics	Tamarix gallica	Antioxidant and antimicrobial	[67]
Phenolics	Marula (Sclerocarrya birrea)	Antioxidants and Antiatherogenic	[68]
Capsaicinoids	Peppers	Antioxidants, anti-mutagenic, anti-inflammatory and anti-tumoral properties	[69]

4.HPLC-NMR[70]

NMR is perhaps the least sensitive spectroscopic method now in use, yet it nevertheless offers the most valuable structural data for understanding the structure of natural compounds. The new practical technology HPLC-NMR or LC-NMR, which has been well-known for more than 15 years, was made possible by technological advancements that permitted the direct parallel coupling of HPLC systems to NMR.

LC-NMR experiments can be performed in both continuous-flow and stop-flow modes. A wide range of bioanalytical problems can be addressed using 500, 600, and 800 MHz systems with 1H, 13C, 2H, 19F, and 31P probes. The continuous-flow probe and a valve installed before the probe for recording either continuous-flow or stopped-flow NMR spectra are the primary requirements for on-line LC-NMR in addition to the NMR and HPLC instrumentation.

In fact, stopped-flow modes, such as time-slice mode, were developed as a result of the advantages of the closed-loop separation-identification circuit and the potential to fully automate the use of all currently existing 2D and 3D NMR techniques. LC-NMR experimentation often done in this manner.

Typically, the LC unit of an LC-NMR system consists of an autosampler, an LC pump, a column, and a non-NMR detector (e.g., UV, DAD, EC, refractive index, or radioactivity). The flow is sent into the LC-NMR interface from this detector, which has extra loops for the temporary storing of chosen LC peaks. Following that, the flow from the LC-NMR interface is directed either to the flow-cell NMR probe-head or to the trash can. The flow is then directed to a fraction collector for recovery and additional research on the distinct fractions evaluated by NMR after passing through the probe-head. At the LC-NMR interface's output, a splitter allows an MS to be connected to the system.

In most of the LC-NMR operations, reversed-phase columns are used, employing a binary or tertiary solvent mixture with isocratic or gradient elution. The protons of the solvents of the mobile phase cause severe problems for obtaining an adequate NMR spectrum.

The development of a suitable LC separation that concentrates the amount of the separated compound in the smallest possible elution volume is essential because the sensitivity of LC-NMR is significantly lower than that of other hyphenated methods, such as LC-MS or LC-PDA. For thorough on-line structural investigation, LC-NMR is a potentially intriguing supplementary approach to LC-UV-MS. In fact, LC-NMR is currently emerging as a potent analytical technique as a result of recent advancements in NMR technology.

Fig 6: HPLC-NMR Instrument.[71]

The Nutraceuticals analysed with HPLC-NMR are given in the following table 4.

Nutraceuticals	Matrix	Health Effects	Ref. No.
Saponins	Vegetables	Stimulate muscle growth and raise testosterone levels. Antidiabetic or anti-obese effects, antibacterial and antineoplastic properties	[72]
Polysaccharide	Poria cocos (fungus)	Anti-inflammatory effects	[73]
Glycosides (glucosinolates, glycyrrhetic acid, glycyrrzhin, liquiritin, steroidal glycosides...)	Plants	Choleretic, anti-inflammatory, anti-cancer, antioxidant, anorexant and diuretic properties	[74]
Resveratrol Oligomers and Flavonoids	Carex folliculata Seeds	Antioxidant, cytotoxicity and antibacterial	[75]
Flavonoids	Citrus peel	Antiinflammatory, anticarcinogenic and antiatherogenic	[76]
Phaeophytines	Amaranthus tricolor (Amaranthaceae)	Antioxidant, cancer prevention	[77]
Lycopene	Tomato products, nutritional supplements	Antioxidant, anti-cancer	[78]

5.GC-MS[79]

The hyphenated technology known as GC-MS, which was created by combining GC and MS, was the first of its kind to be effective for research and development. Based on the interpretation of fragmentations, mass spectra generated using this hyphenated approach

provide additional structural information. The library spectra of the fragment ions with various relative abundances can be compared. By using GC-MS, it is simple to evaluate substances that are sufficiently volatile, tiny, and stable at high temperatures in GC settings. For GC-MS analysis, polar molecules, particularly those with a lot of hydroxyl groups, occasionally need to be derivatized.

The process of changing the analyte into its trimethylsilyl derivative is the most popular derivatization method. GC-MS involves injecting a sample into the GC device's injection port, vaporising it, separating it in the GC column, analysing it with an MS detector, and recording the results. Retention time is the period of time between injection and elution. Injection ports at one end of a metal column that is usually packed with sand-like material to facilitate maximum separation and a detector (MS) at the other end of the column are the main components of GC-MS equipment.

The sample is propelled down the column by a carrier gas (such as argon, helium, nitrogen, or hydrogen, to mention a few). The MS detector offers information that aids in the structural identification of each component, whereas the GC separates the components of a mixture over time. Capillary columns and macrobore and packed columns are the two types of GC-MS columns that are available.

The following points need to be considered carefully regarding the GC-MS interface.

1. The interface transports efficiently the effluent from the GC to MS.
2. The analyte must not condense in the interface.
3. The analyte must not decompose before entering the MS ion source.
4. The gas load entering the ion source must be within the pumping capacity of the MS.

The chemical ionisation (CI) and electron impact ionisation (EI) modes are the two interfaces for a GC-MS that are most frequently employed. Modern GC-MS systems, however, allow for the use of a number of different types that enable molecular ion identification. For instance, by measuring exact mass and determining elemental composition, an orthogonal TOF mass spectrometry coupled with GC is utilised to certify the purity and identification of the components. A GC-MS is now integrated with multiple online MS databases for a number of reference compounds, and these databases have search capabilities that could be helpful for spectra matching for the identification of separated components.

Fig 7: GC-MS Instrument.[80]

The Nutraceuticals analysed with GC-MS are given in the following table 5.

Nutraceuticals	Matrix	Health Effect	Ref. No.
Sterols	Mediterranean mussel and Rapana venosa (hard-shellclam)	Skin-care	[81]
Terpenes and terpenoids	Essential oils	Antiseptic, carminative, antimicrobial, and antioxidative effects.	[82]
Phytosterols and phytostanols	Milk and yoghurt	Decrease cholesterol levels	[83]
Saponins	Vegetables	Stimulate muscle growth and raise testosterone levels. Antidiabetic or anti-obese effects, antibacterial and antineoplastic properties	[84]
Phenolic acids	Malt	Antioxidant	[85]
Catechins and condensed tannins	Green Tea	Antioxidant	[86]
Leuconostoc paramesenteroides	cheddar cheese	-	[87]
Phenolic acids	Mangosteen	Antioxidant	[88]

6.GC-FID[89]

One of the most used detectors for gas chromatography is the flame ionisation detector (FID). The scope of application is extensive. For the conversion of energy, the kerosine's composition is crucial. The packing of food is a very separate topic. Several hydrocarbons are added to polystyrene during processing to produce the finished product. When polystyrene is used in the food sector, it is essential to analyse the product for any

hydrocarbon residues because they might affect the food's quality and be harmful to your health.

The FID is well suited for analysis of hydrocarbons but also for organic substances containing hydrocarbons and for volatile organic compounds. The sample is burned in a FID using a hydrogen/synthetic air flame. In the flame, ions and free electrons are created. The detector's gap between its two electrodes experiences a measurably high current flow as a result of the charged particles. The signal created by the pure carrier gas and the fuel gas flame alone is weaker than the current flow that results. The differential signal tells us something about the sample. The ion formation, which depends on the makeup of the separated sample, determines how much current flows.

The FID is a general detector that can be used to analyse more specialised components after being further configured. Carbon-containing components, for instance, can catalytically convert to methane and become acceptable for FID analysis by being placed in front of a methaniser. This method is frequently used to analyse carbon monoxide (CO) and carbon dioxide (CO2). A new FID configuration is required for the detection of organic nitrogen/phosphorus compounds. The sample travels via a hot alkali source, where charged particles are created when the source comes into touch with the sample. The term "thermionic detection" is also used to describe this technique, which is also known as "alkali flame ionisation." The type of detector employed for this technique is one that uses thermal energy as the source of ionisation. Nitrogen/phosphorous detection is another name for this technique, and NPD stands for the corresponding detector. Flame ionisation detectors have a large linearity range and are quite sensitive. The sample they receive is the sole drawback for them.

The employment of a carrier gas to move the sample from the injector through the column and into the FID is a crucial component of the FID. The column material cannot absorb the carrier gas, which must be inert. As carrier gases for the FID, helium or nitrogen are often employed, with hydrogen occasionally being added. During the combustion process, hydrogen and synthetic air, the detector gases, act as fuel gas and oxidising gas, respectively. Hydrocarbon contaminants, moisture, and oxygen in the detector gases should be kept as low as possible since they increase baseline noise, which has a negative impact on the detection limit.

Fig 8: GC-FID Instrument.[90]

The Nutraceuticals analysed with GC-FID are given in the following table 6.

Nutraceuticals	Matrix	Health effect	Ref. no.
Sterols	Italian walnut	Decrease cholesterol and reduce the risk of coronary heart disease	[91]
Glycerolipids	Seed oils	Skin care and source of fatty acids	[92]
Plant sterols (Phytosterols)	Vegetable oils (olive, sunflower, rice bran, seeds,...)	Phytosterols decrease cholesterol associated with LDL, have anti-cancer activity and modulate the immune function and inflammation.	[93,94]
Tocopherols (Vitamin E)	Vegetable and vegetable oils	Antioxidant, antitumor, hypocholesterolemic potential and for the treatment of cardiovascular disease and angiogenic disorders	[95,96,97]
Galactooligosaccharides	Dairy-based prebiotic ingredient.	increased absorption of calcium and magnesium, and improved elimination of toxic compounds	[98]
Squalene	Vegetable oil	Decrease cholesterol and anti-cancer activity	[99]

7.HPTLC (High Performance Thin Layer Chromatography)[100]

Steps involved in HPTLC

Selection of chromatographic layer

Precoated plates - different support materials - different Sorbents available 80% of analysis - silica gel GF · Basic substances, alkaloids and steroids - Aluminum oxide Amino acids, dipeptides, sugars and alkaloids - cellulose Non-polar substances, fatty acids, carotenoids, cholesterol - RP2, RP8 and RP18 Preservatives, barbiturates, analgesic and phenothiazines- Hybrid plates-RPWF254s.

Sample and Standard Preparation

To avoid interference from impurities and water vapours Low signal to noise ratio - Straight base line- Improvement of LOD Solvents used are Methanol, Chloroform: Methanol (1:1), Ethyl acetate: Methanol (1:1), Chloroform: Methanol: Ammonia (90:10:1), Methylene chloride : Methanol (1:1), 1% Ammonia or 1% Acetic acid Dry the plates and store in dust free atmosphere

Activation of pre-coated plates

Plates in a recently opened box don't need to be activated. Plates that have been on hand for a while or are highly humid will activate 30 minutes in an oven preheated to 110–120°C before spotting Aluminum sheets should be placed between two glass plates and baked for 15 minutes at 110–120 °C.

Application of sample and standard

A concentration range above the typical range of 0.1 to 1 g/l results in poor separation. Nitrogen gas is sprayed automatically from a syringe onto TLC plates to create bands from the sample and the standard. improved separation with band-wise application high densitometer response.

Selection of mobile phase

Trial and error

Ones own experience and Literature based

Mainly two types: Normal Phase (Stationary phase is polar)

Reverse phase (Stationary phase is Non-polar)

Conditioning prior (Chamber saturation)

Rf values are high when the chamber is not saturated. Prior to development, the chamber was saturated by covering it with filter paper for 30 minutes. This resulted in an even distribution of solvent vapours, less solvent for the sample to travel through, and lower Rf values.

Drying and chromatographic development

To avoid contaminating the lab environment, remove the plate after development and remove the mobile phase from the plate. Avoid using a hair dryer when drying with vacuum desiccators since the essential oil components may evaporate.

Detection and visualization

First-choice detection under UV light is nondestructive and fluorescent compound spots can be visible at 254 nm or 366 nm (short wave length) (long wave length) Patches of non-fluorescent substances can be visible; silica gel GF is employed as the fluorescent stationary phase. Ethambutol and dicylomine, which don't absorb UV light, are used, as well as immersing the plates in a 0.1% iodine solution When a certain component is not UV-responsive, derivatization is necessary for detection.

Quantification

Sample and standard should be chromatographically separated on the same plate. After development, the chromatogram is scanned using a TLC scanner III in reflectance, transmittance, absorbance, or fluorescent mode. Scanning speeds can be adjusted up to 100 mm/s, and 36 tracks with up to 100 peak windows can be quickly recorded. It is feasible to calibrate single and multiple levels using linear or non-linear regressions. Single level calibration is appropriate when goal values need to be verified, such as during stability testing and dissolution profile. When using statistics like RSD, the concentration of the analyte in the sample is estimated by taking into account both the initial sample and dilution variables.

Fig 9: HPTLC Instrument[101]

The Nutraceuticals analysed with HPTLC are given in the following table 7.

Nutraceuticals	Matrix	Health effect	Ref. No.
Gangliosides	Dairy products (milk)	Protect against enteric pathogens, and prebiotic functions.	[102]
Lycopene	Tomato products, nutritional supplements	Antioxidant, anti-cancer	[103]
Vitamins B2, B3 and B6	Energy drinks	Antioxidant and co-enzymes	[104]
Curcuminoids	Curcuma longa	Antioxidant	[105]
Phenolics	Vanilla planifolia	Antioxidant	[106]

CONCLUSION

In this work, we have presented an overview on nutraceuticals covering the recent time period, discussing the different bioactive compounds (lipids, vitamins, proteins, glycosides, phenolic compounds), their claimed health promoting effects, the analytical techniques mainly employed for their analysis and the natural matrices in which they are found.

It can be concluded that the preferred analytical tools for analysing bioactive compounds are GC and HPLC, probably due to their versatility, generalized availability, low-cost and simplicity. It must be pointed out that practically all the claimed health promoting effects of the bioactive compounds presented in this work, have not been recognized yet by the pertinent authorities (EFSA, FDA, etc.), because in most of the cases there is a lack of long term studies and/or clinical trials that demonstrate unquestionably their health effects.

In this sense, it is highly required the development and application of advanced analytical approaches as the new Foodomics strategy. in order to obtain superior information on nutraceuticals. This new information should make easier the identification in natural matrices of new nutraceuticals, their chemical characterization and the unquestionable confirmation of their health promoting effects when combined with well designed clinical trials.

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