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High Performance Liquid Chromatography

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Abstract

The OPA method has been optimised, making separation and quantification of 23 amino acids possible, including 5 frequently searched for. Detection limits in honey ranged from 0.24 to 10.1 pmol and in bee-pollen from 29.1 to 0.42 pmol; reproducibility (C.V.) ranged from 5.3% to 20.4%; and recoveries were greater than 78.8%. The free amino acid profiles of forty monovarietal honey samples from ilex, oak, heather, and chestnut-tree were determined. Honey contains a-aminoadipic acid and homoserine for the first time.

Keywords

Amino acids; Honey; Bee-pollen;HPLC

Introduction

The techniques through which the chemical components present in complex mixtures are Separated, identified, and determined is termed chromatography. This technique is widely used like spectroscopy and is a very powerful tool not only for analytical methods but also for preparative methods. Compounds of high-grade purity can be obtained by this method. Chromatography can be simply defined as follows: “It is the technique in which the components of a mixture are separated based upon the rates at which they are carried or moved through a stationary phase (column) by a gaseous or liquid mobile phase”.

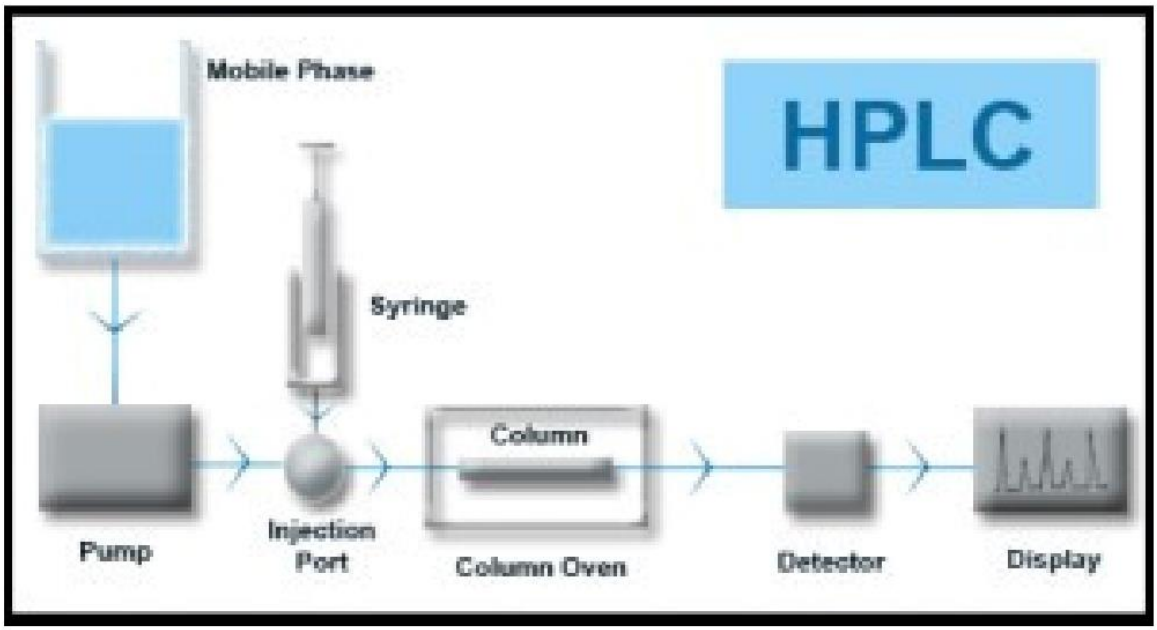
Chromatography is the most widely used laboratory technique for separation, identification and quantification of components of liquid and gaseous mixtures. Solid mixtures are also analyzed by first converting them to a liquid or gaseous state, using suitable sample preparation techniques. Differential affinity of components between the carrier and stationary phases forms the basis of separation. Components retained by stationary phase have slower migration rates than unretained or partially retained components. You can think of the molecular mixture as a family passing by a candy store which is the stationary phase. The children will tend to get retained because of their affinity for candy,

while the parents will keep on moving like un-retained molecules leading to a separation between children and parents!Various techniques have been adapted to identify and quantify the components that migrate through the chromatographic system in a sequence depending on operating parameters. We will be discussing these detectors in-depth in chapter number 6.

In earlier days liquid mobile phase was commonly used in paper, thin layer and column chromatography. In paper and thin layer chromatography separation of components takes place as the solvent moves along the filter paper or coated plates by capillary action. In column chromatography an empty glass tube is packed with finely powdered stationary phase and a small volume of liquid mixture is applied to top of column. Mobile phase liquid is continuously added which elutes sample components sequentially based on affinity with stationary phase. The individual components can be detected visually or with a detection system.

In Gas chromatography same principle is used but carrier is a gas instead of a liquid. The stationary phase is an immobilized liquid bound to an inert support or simply applied to the inner surface of a column. Gas chromatography is effectively used for analysis of gas mixtures or liquids having low boiling points. On the other hand liquid chromatography is applied for separation of thermally labile liquid mixtures or those having high boiling points.

Size exclusion chromatography is based on separation of molecules on the basis of their size .Stationary phases are selected on basis of their pore size and selective retention takes place depending on the pore size of stationary phase .No chemical interaction takes place between the stationary phase and the eluting species. Liquid Chromatography has played a key role in development of HPLC. The



next chapter takes you through the evolution of Liquid Chromatography to its present day status.

High Performance Liquid Chromatography and its parts

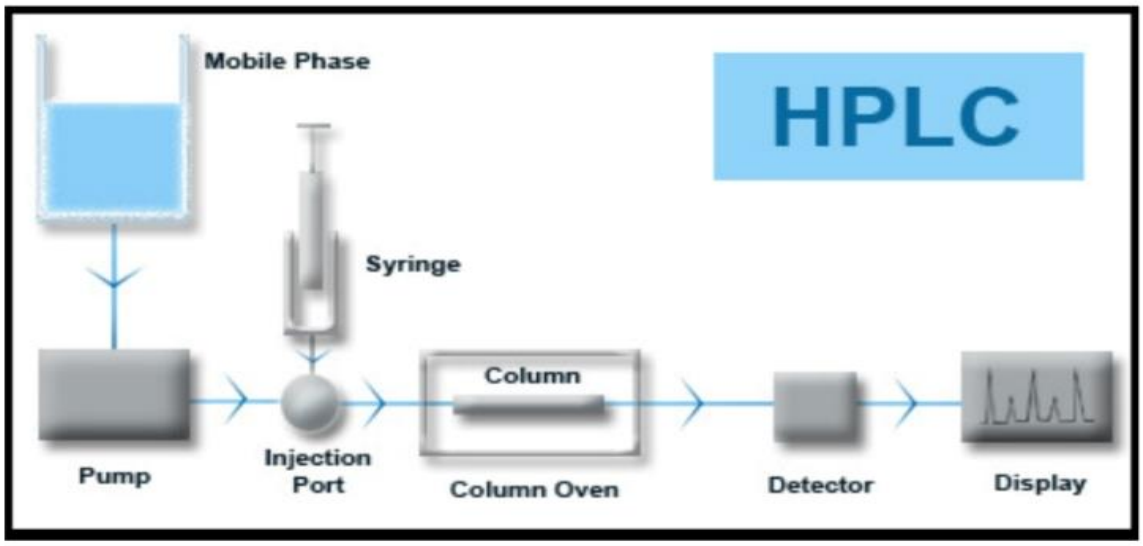
Chromatography equipment look rather intimidating to anyone who has not handled them before, but on a closer look and as you get familiar with the equipment you realize that behind the network of wires, complex plumbing and circuitry is a simple machine with only a few major
parts. Different combinations of these parts namely pumps, detectors and injectors yield an
infinite number of configurations based on the application. Just like an understanding of human anatomy makes you conscious of the vital role of each and every body organ towards your well being and vitality. Similarly you need to have a good understanding of the parts of your HPLC system to generate data of highest reliability. A conceptual understanding of the function of each component will add to your comfort level with your HPLC system. You will ensure long time usage with high reliance on output data. The present chapter is intended to serve this very purpose and in simple terms you will appreciate the role of each part and its contribution to overall system efficiency.

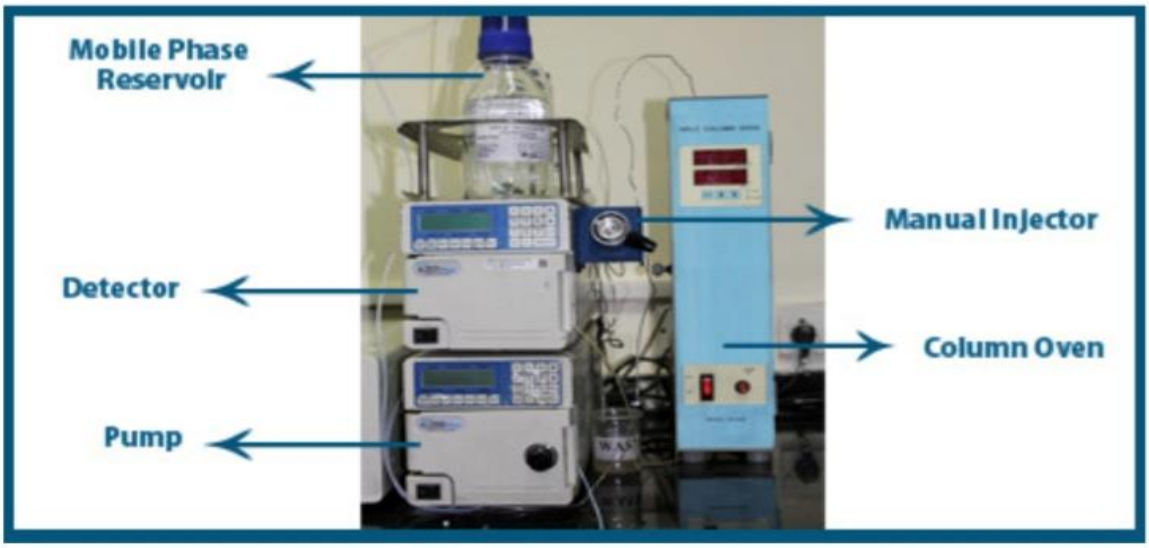
HPLC is a technique for separation, identification and quantification of components in a mixture. It is especially suitable for compounds which are not easily volatalised, thermally unstable and have high molecular weights. The liquid phase is pumped at a constant rate to the column packed with the stationary phase. Before entering the column the analysis sample is injected into the carrier stream. On reaching the column the sample components are selectively retained on the basis of physico-chemical interactions between the analyte molecules and the stationary phase. The mobile phase moving at a steady rate elutes the components based on the operating conditions. Detection techniques are employed for detection and quantification of the eluted components. We now introduce you to the significance and role of each component part of the HPLC system.

Mobile PhaseMobile phase serves to transport the sample to the system. Essential criteria of mobile phase are inertness to the sample components. Pure solvents or buffer combinations are commonly used. The mobile phase should be free of particulate impurities and degassed before use.

Mobile Phase Reservoirs

These are inert containers for mobile phase storage and transport. Generally transparent glass
bottles are used so as to facilitate visual inspection of mobile phase level inside the container.
Stainless steel particulate filters are provided inside for removal of particulate impurities in the
Mobile phase if any.





Pumps

Variations in flow rates of the mobile phase effect elution time of sample components and result in errors. Pumps provide constant flow of mobile phase to the column under constant pressure.

Injectors

Injectors are used to provide constant volume injection of sample into the mobile phase stream.

Inertness and reproducibility of injection are necessary to maintain high level of accuracy.

Column

A column is a stainless steel tube packed with stationary phase. It is a vital component and should be maintained properly as per supplier instructions for getting reproducibility separation efficiency run after run.

Column Oven

Variation of temperature during the analytical run can result in changes of retention time of the separated eluting components. A column oven maintains constant column temperature using air circulation. This ensures a constant flow rate of the mobile phase through the column

Detector

A detector gives specific response for the components separated by the column and also provides the required sensitivity. It has to be independent of any changes in mobile phase composition. Majority of the applications require UV-VIS detection though detectors based on other detection technique are also popular these days.

Data Acquisition & Control

Modern HPLC systems are computer based and software controls operational parameters such as mobile phase composition, temperature, flow rate, injection volume and sequence and also acquisition and treatment of output.

These are the main parts of a basic HPLC system more specialized equipment might also have solvent selection valves, vacuum degasser, auto samplers, column switchers, pre or post column derivatization and fraction collectors. These are all covered at length in our upcoming online certificate program on HPLC.

The first three chapters have covered general course introduction, evolution of chromatography and a brief introduction to HPLC component parts. After going through these chapters you would have felt an urge to know more about this popular technique.

You'll be pleased to know that an elaborate certificate programme is also available online. The certificate program is designed to provide you exposure to the technique through 18 sequential steps.To find more click on the link http://lab-training.com/product/join-our-certificate-course-on-hplc/The next chapter will introduce you to the types of stationary phases used in columns for separation of compounds.

Types of Mobile Phases

In the previous chapter you were introduced to the stationary phases which serve to retain

selectively the sample components. After separation these components need to be transported to the detector for detection and quantification. Mobile phase is the life line of HPLC system as it transports the sample from the injector to the detector and its characteristics such as composition, pH etc, have a profound effect on separation of sample components. The mobile phase should have the following desirable characteristics to carry out this important function.

• Affordable cost

• Non hazardous

• Inertness towards sample constituents and stationary phase

• Sample components should be miscible fully with the mobile phase

• Detector should not respond to mobile phase or to changes in the mobile phase

composition as in gradient elution Mobile phases generally consist of water – organic solvent, aqueous buffer or mixtures of organic solvents with or without modifiers. In isocratic mode the composition of the mobilephase remains unchanged throughout the analytical run whereas in the gradient elution mode mobile phase composition changes through programming of the pump. Choice of mobile phase depends on the mode of HPLC operation.

Mobile Phases in Reverse Phase chromatography

In reverse phase applications water is usually the base solvent. Other polar solvents such as Methanol, Acetonitrile or Tetrahydrofuran are added in fixed or varying proportions. pH is adjusted by buffers to modify separations of ionizable solutes. Ion-pairing reagents also enhance separation selectivity of charged analytes by increasing retention on hydrophobic bonding phases.

Mobile Phases in Normal Phase Chromatography

Typically non polar solvents such as hexane, heptane, iso-octane are used in combination with slightly more polar solvents such as isopropanol, ethyl-acetate or chloroform. Retention increases as the amount of non polar solvent increases in the mobile phase.

Mobile Phases in Ion Exchange Chromatography

Aqueous salt solutions are generally used as mobile phases. Moderate amounts of water miscible polar organic solvents such as methanol can be added to buffered mobile phases. Solvent strength and selectivity can be adjusted by control of pH, buffer and salt concentrations. Mobile Phases for Size Exclusion ChromatographyThe mobile phase composition is not varied as the detector is sensitive to such changes. Choice of mobile phase is dependent on its ability to dissolve sample and maintain consistent viscosity at operating temperature. High polarity solvents such as acetone, alcohols, DMSO and water are not used with polystyrene packings. Ionic strength is maintained by addition of salts

Types of Detectors

A detector can be compared to a gate watchman who verifies the visitors before permitting them entry inside a building. The chromatographic detector is capable of establishing both the identity and concentration of eluting components in the mobile phase stream. A broad range of detectors is available to meet different sample requirements. Specific detectors respond to a particular compound only and the response is independent of mobile phase composition. On the other hand the response of bulk property detectors is dependent on collective changes in composition of sample and mobile phase.

The desirable features of a detector are:

- Sensitivity towards solute over mobile phase
- Low cell volumes to minimize memory effects
- Low detector noise
- Low detection limits
- Large linear dynamic range

UV-VIS Detector

UV-VIS Detector is the most commonly used detector. Its response is specific to a particular

compound or class of compounds depending on the presence of light absorbing functional groups of eluting molecules. Some compounds which do not have such light absorbing groups can give suitable response after post column derivatization to introduce light absorbing entities

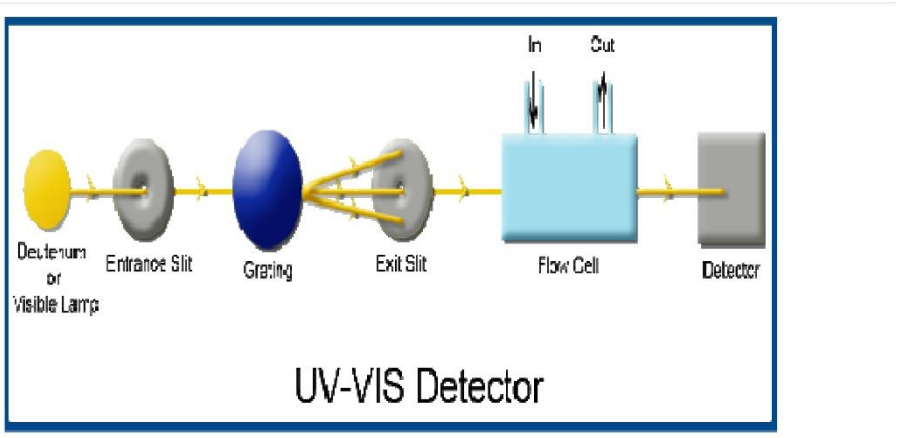


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Photo Diode Array Detector

Incorporation of large number of diodes which serve as detector elements makes possible simultaneous monitoring of more than one absorbing component at different wavelengths. This provides benefit of time saving and cost reduction on expensive solvents.

Fluorescence Detector

Fluorescence detection offers greater sensitivity than a UV-VIS detector. However, the number of naturally fluorescent compounds is smaller in comparison to light absorbing compounds. This limitation is overcome by post column derivatization.

Mass Spectroscopic Detector

Mass spectroscopy offers very high sensitivity and selectivity. Detection is based on fragmentation of molecules by electric fields and separation on basis of mass to charge ratios of fragmented molecules. LC-MS technique has opened up new application areas due to advantages of resolution and sensitivity.

Bulk Property Detectors

Refractive Index Detector

The response is dependent on changes in refractive index of eluting compounds in the mobile phase. The mobile phase itself should have refractive index different from the sample. Gradient programming is not possible due to resulting changes in refractive index of mobile phase. The detector is less sensitive than UV-VIS detector. Temperature control is necessary as it has high temperature sensitivity. Typical applications are in Size Exclusion Chromatography.

Electrochemical Detector

Based on electrochemical oxidation or reduction of sample on electrode surface. It is, however, sensitive to changes in composition or flow rate of mobile phase.

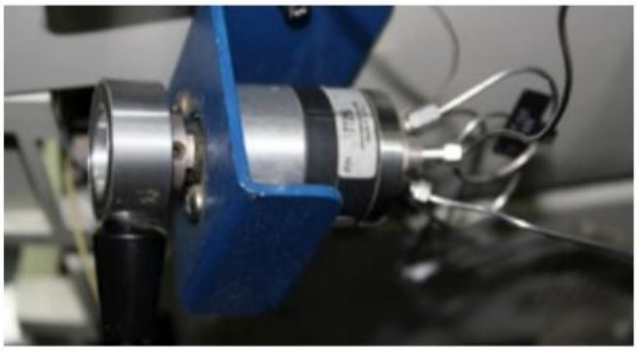
Light Scattering Detectors

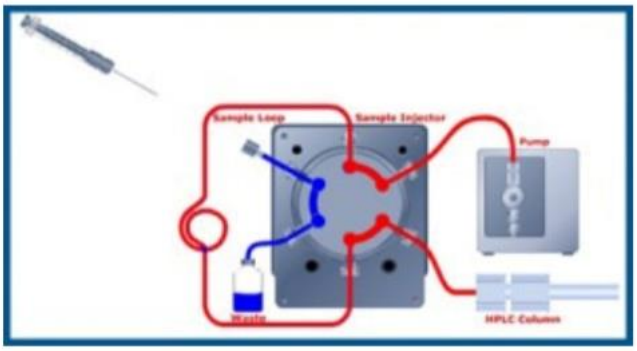
Light scattering detectors are useful for detection of high molecular weight molecules. After removal of mobile phase by passing through a heated zone the solute molecules are detected by light scattering depending on molecular sizes.

Types of High Performance Liquid Chromatography Injectors

Understanding of injectors is as important as that of pumps in the last session. Injectors serve to introduce required sample volume accurately into the HPLC system. Sample injection into the moving mobile phase stream in HPLC is quite different from injection into a gas stream in Gas Chromatography as precise injection is required against high back pressure. In such a situation it is not possible to simply inject using a syringe alone.

Manual injection(Rheodyne/Valco injectors)





Injection is done through specially designed 6-port rotary injection valve. The sample is introduced at atmospheric pressure by a syringe into a constant volume loop. In the LOAD position the loop is not in the path of the mobile phase. By rotating to the INJECT position the sample in the loop is moved by the mobile phase stream into the column. It is important to allow some sample to flow into waste from loop so as to ensure there are no air bubbles in the loop and previously used sample is completely washed out to prevent memory effects.

Automatic Injection Automatic injection improves laboratory productivity and also eliminates personal errors.

Present day advanced HPLC systems are equipped with an auto injector along with an auto sampler. The software programmes filling of the loop and delivery of the sample to the column. The computer also controls the sequence of samples for injection from vials kept in numbered positions of the auto sampler. It is important to adopt precautions to ensure consistency of results and also prolong the service life of the automated system.

• Prime injector with solvents to be used but it should be ensured that solvent is compatible with solvent used earlier.

• Needle wash between samples will prevent carry over between injections.

• Before start and at end of analysis ensure tubing is completely washed of buffers or previously used solvents.

• Do not forget to feed the vial number correctly on auto sampler rack and list out the sequence correctly in the computer.

We have now gained an understanding of HPLC and its systems. The next session will discuss some application areas particularly in analysis of Pharmaceuticals and Foods.

Applications of High Performance Liquid Chromatography

After having gained exposure to High Performance Liquid Chromatography systems and their components we now introduce to typical applications. HPLC has contributed to analytical solutions in diverse fields such as pharmaceuticals, foods, life sciences, environment, forensics, etc. In the present chapter we shall discuss some application areas in pharmaceuticals and foods.

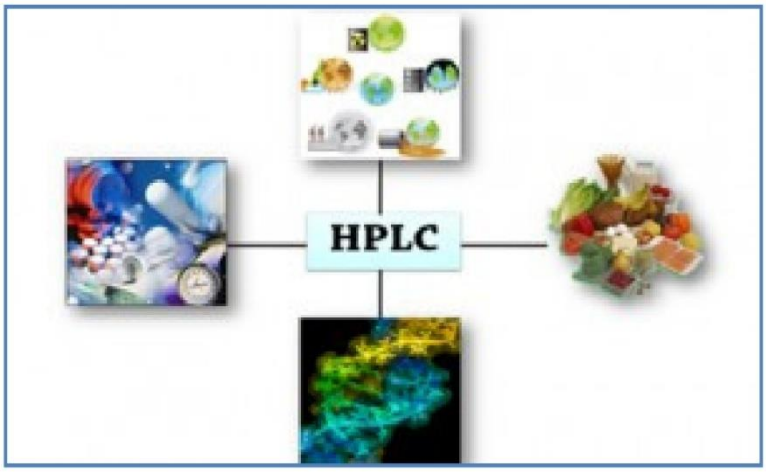
Pharmaceuticals High Performance Liquid Chromatography provides reliable quantitative precision and accuracy along with a high linear dynamic range to allow determination of API and related substances in a single run. A convenient method for sample preparation for solid dosage forms is dispersion in water or aqueous media modified with acetonitrile or methanol .HPLC offers several possibilities for separation of chiral molecules into their respective enantiomers.These include precolumn derivatization to form diastereomers. Alternately, specialty columns prepared with cyclodextrins or special chiral moieties as stationary phases maybe used .In short HPLC, particularly reverse phase HPLC is the most popular choice for quantitative analysis in the pharmaceutical industry.

Common application areas in pharmaceutical analysis are :

- Assay
- Related Substances
- Analytical Method Validation
- Stability Studies
- Compound Identification
- Working Standards

Foods

High Performance Liquid Chromatography has brought desirable advantages in the field of food analysis. Food matrices are generally complex and extraction of analytes is not an easy task. To further complicate matters both desirable and undesirable components are often found in trace levels and classical extraction and analysis does not provide the required levels of accuracy and



precision. HPLC offers viable solutions due to vast choice of stationary phases and mobile phase options. Common applications in foods are :

- Fat soluble vitamins (A,D,E and K)
- Water soluble vitamins (B-complex vitamins such as B1, B2, B3, B6, Folic acid, Pantothenic acid, B12, VitaminC)

- Residual pesticides such as 2, 4-D and Monochrotophos.
- Antioxidants such as TBHQ, BHA and BHT.
- Sugars: Glucose, Fructose, Maltose and other saccharides
- Cholesterol and sterols
- Dyes and synthetic colours.
- Mycotoxins such as Aflatoxins B1,B2,G1,G2,M1,M2and ochratoxin
- Amino acids
- Residual antibiotics
- Steroids and flavanoids
- Aspartame and other artificial sweeteners.
- Active ingredients of farm produce such as allin in garlic and catachin in tea extracts.[1]

HPLC Basics – principles and parameters

PRINCIPLE OF HPLC

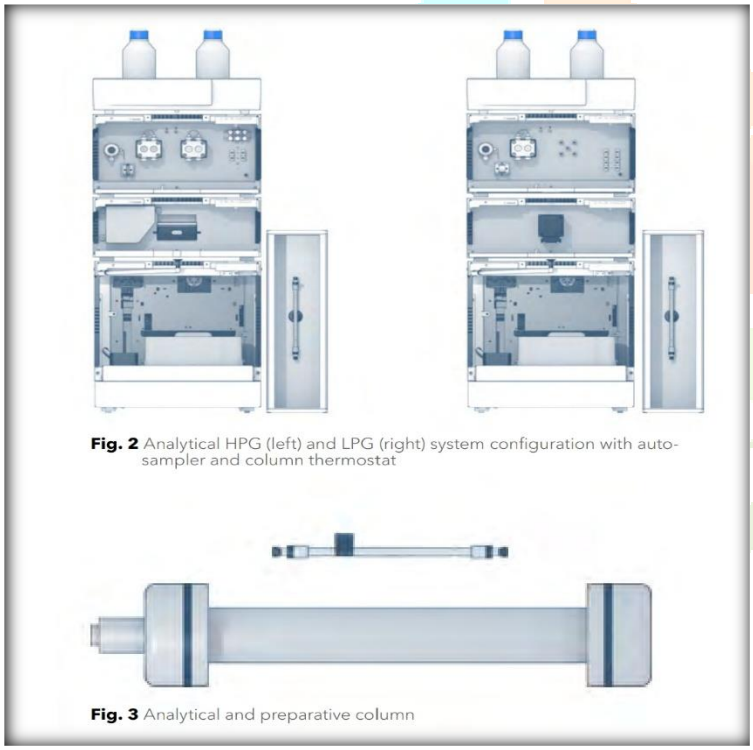


Fig. 2 Analytical HPG (left) and LPG (right) system configuration with auto-sampler and column thermostat

Fig. 3 Analytical and preparative column

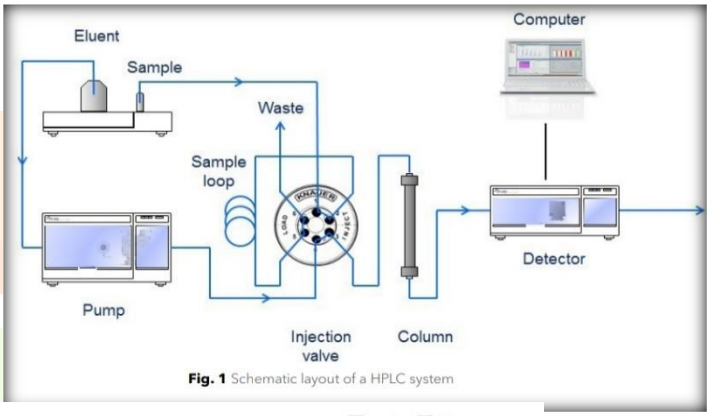


Fig. 1 Schematic layout of a HPLC system

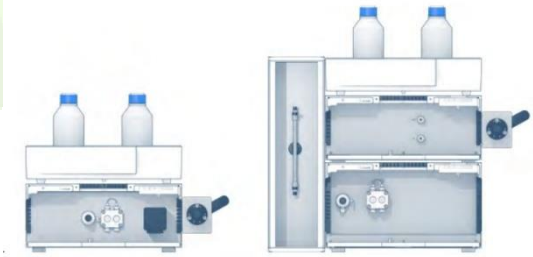


Fig. 4 Isocratic system configuration with a single wavelength UV-detector (left) and a refractive index detector (right)

The separation principle of hplc is based on the distribution of the analyte (sam-
Ple) between a mobile phase (eluent) and a stationary phase (packing material of
The column). Depending on the chemical structure of the analyte, the molecules
Are retarded while passing the stationary phase. The specific intermolecular inter-
Actions between the molecules of a sample and the packing material define their
Time “on-column”. Hence, different constituents of a sample are eluted at different
Times. Thereby, the separation of the sample ingredients is achieved. A detection
Unit (e.g. uv detector) recognizes the analytes after leaving the column. The signals
Are converted and recorded by a data management system (computer software)
And then shown in a chromatogram. After passing the detector unit, the mobile
Phase can be subjected to additional detector units, a fraction collection unit or
To the waste. In general, a hplc system contains the following modules: a solvent
Reservoir, a pump, an injection valve, a column, a detector unit and a data proces-

Sing unit (fig. 1). The solvent (eluent) is delivered by the pump at high pressure and Constant speed through the system. To keep the drift and noise of the detector si-Gnal as low as possible, a constant and pulseless flow from the pump is crucial. The Analyte (sample) is provided to the eluent by the injection valve.

Depending on the composition of the mobile phase, two different modes are generally applicable. If the makeup of the mobile phase remains constant during the separation process, the HPLC system is defined as an isocratic elution system. When the composition of the mobile phase is changed during separation, the HPLC system is defined as a gradient elution system. [2,3] Using a gradient system, two different techniques are available: a low-pressure gradient (LPG) and a high-pressure gradient (HPG). A low-pressure gradient means that the mixing of the solvents is carried out upstream of the pump (suction side). In a high-pressure gradient system, the different solvents are supplied by individual pumps and mixed after the pumps (discharge side). [2] Fig. 2 shows exemplary system configurations for a LPG and a HPG gradient mode.

Column

The column represents the heart of any HPLC system. It is responsible for the adequate separation of the sample ingredients. The separation efficiency correlates with the column inner diameter, the length of the column and the type and particle size of the column packing material. Depending on the desired application, numerous HPLC columns are commercially available. Different packing materials support different separation mechanisms – common are materials for normal-phase, reversed-phase, size exclusion, ion exchange, affinity, chiral, or hydrophilic interaction HPLC. [2] In Fig. 3 an analytical and a preparative column are shown.

Detector

The task of the detector unit is to register the time and amount of a substance which is eluted from the column. The detector perceives the change in the composition of the eluent and converts this information into an electrical signal which is evaluated by the aid of a computer. [2] A variety of detectors is available depending on the structural characteristics of the analyte. Common detector units are refractometric, UV/VIS, electrochemical and fluorescence detectors. Fig. 4 shows two isocratic system configurations with different detectors.

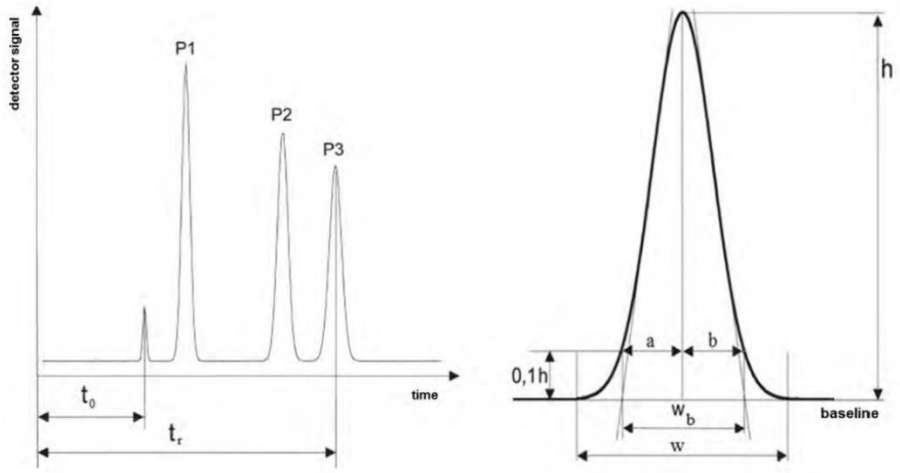


Fig. 5 Schematic illustration of a chromatogram [4]

Chromatographic parameters

The separated analytes which are transported by the mobile phase are recorded as signal peaks by the detector unit. The total amount of all peaks is called chromatogram. Each individual peak provides qualitative and quantitative information of the analyte. Qualitative information is given by the peak itself (e.g.: shape, intensity of the signal, time of appearance in the chromatogram). In addition, the area of a peak is proportional to the concentration of the substance. Hence, the chromatography data management software can calculate the concentration of the sample by integration. This provides quantitative information. Ideally the peaks are recorded as a Gaussian bell-shaped curve. A schematic example is illustrated in Fig. 5. The basic parameters of a chromatographic sepa-ration are discussed below.

Delay time (t0)

The delay time refers to the time which is required for a non-retarded compound to be transported from the injection site to the detector unit (where the com-pound is recorded). During this time, all sample molecules are exclusively located in the mobile phase. In general, all sample molecules share the same delay time. The separation is caused by differing adherence of the substances with the stationary phase.

Retention time (tR)

The retention time refers to the time which is required for a compound from the moment of injection until the moment of detection. Accordingly, it represents

the time the analyte is in the mobile and stationary phase. The retention time is substance-specific and should always provide the same values under the same conditions.Peak width (w) The peak width covers the period from the beginning of the signal slope until reaching the baseline after repeated drop in the detector signal.

Tailing factor (T)

In practice, perfectly symmetric peaks are very rare. In a chromatogram they often show some degree of tailing. Peak tailing is measured by the tailing factor T. This factor describes the peak asymmetry, i.e. to which extent the shape is approximated to the perfectly symmetric Gaussian curve. The tailing factor is measured as: T=b/aa represents the width of the front half of the peak, b is the width of the back half of the peak. The values are measured at 10 % of the peak height from the leading or trailing edge of the peak to a line dropped perpendicularly from the peak apex (see Fig. 5). [4]T = 1 represents a symmetrical peak. For T > 1 the peak profile is named tailing. For T < 1 the peak profile is named fronting.

PROPERTIES OF SOLVENTS USED IN HPLC

Chromatographers have a choice among hundreds of solvents for use as mobile-phase components, sample solvents, or in sample pretreatment. A particular selection is usually affected by solvent characteristics that relate to detection, separation, flow resistance (column pressure drop or mobile phase viscosity), and miscibility. Commercial availability in adequate purity and at a reasonable price are also important factors. The solvent properties described in this appendix will be useful to chromatographers when it comes to selecting one or more solvents.

II.1 DETECTION

The choice of mobile-phase solvent can have a profound effect on the ease and sensitivity of HPLC detection. The lowest usable (cutoff) wavelength is important for UV detectors (Tables 3.2 and 3.3), solvent refractive index (RI) affects the sensitivity of RI detection for a particular sample, and solvent volatility (boiling point) is important for evaporative light-scattering detectors. Table II. 1 summarizes these and other properties that may affect detection for a number of common solvents. Additional data are provided for most HPLC-grade solvents in RRefs.[5](e.g., complete UV spectra for most HPLC-grade solvents, blank gradients for some A- and B-solvent combinations, etc.).

II.2 SEPARATION

Mobile-phase solvents can affect separation by their polarity and selectivity.

More polar solvents cause increased retention in RPC and reduced retention

Current practice of HPLC-MS in metabonomics

As indicated in Section 1, the application of HPLC-MS formetabonomic studies is relatively new. Such studies, as have been published, include the investigation of toxicity in rats byPlumb et al. [6], Idborg-Bjorkman et al. [7] and Lafaye etal. [8] and metabotyping (metabolic fingerprinting) [12] of strain, gender and diurnal variation in mice Plumb et al. [9].In our own studies, we have used gradient reversed-phase HPLC-orthogonal acceleration (oa) -time-of-flight (TOF) -MS and MS/MS for the examination of urine from various strains of rat (in preparation) and mouse [9] and from rats

exposed to a number of nephrotoxins [10,11]. In these stud-ies, a simple linear gradient has usually been applied withthe samples analysed using both +ve and −ve electrospray ionisation (in separate analytical runs). In a typical experiment, a 10l aliquot of urine was injected onto a 2.1 mm ×10 cm Symmetry® C18 3.5m HPLC column. The columnwas maintained at 40 ◦C and elution was performed with alinear gradient of 0.1% aqueous formic acid to 20% acetonitrile (containing 0.1% formic acid) over the period 0.5–4 min.This was followed by an increase in the acetonitrile content to95% over the period 4–8 min. After holding the solvent composition at 95% acetonitrile for a further minute the column was then returned to its starting conditions. An eluent flowrate of 600 l /min was used and mass spectrometric data was collected over the mass range of 50–850, in either positive ornegative ion mode on a time-of-flight mass spectrometer. The column eluent was split such that approximately 120 l/minwas directed to the mass spectrometer.As part of our investigations into the utility of HPLC-MS,we elected to study the effects of strain and gender on the uri-nary metabolic profiles of black, white and nude mice [9].Samples were collected in both the morning and the afternoon so that we could also examine diurnal variation, as in previous NMR-based studies, differences were readily observed for both strain and collection time [12,13]. Mice represent an important experimental animal for the discovery of new drugs, with the nude mouse being especially important for the development of anticancer compounds. An understand-ing of typical “baseline” physiological variation is therefore an essential pre-requisite for in-depth metabonomic studies in drug discovery and development. Typical positive and negative ion total ion current chromatograms (TICs) for a morning sample obtained from a black male animal are shownin Fig. 1. Such profiles contain many hundreds of individual components rendering visual comparison complex. However,by using pattern recognition approaches based on principal components analysis (PCA), it was possible to show that all

three strains of mouse could be separated from each other based on their MS-detected metabolite profiles. A typical example of such PCA analysis is shown in Fig. 2, for black, nude and white male mice, where the scores plot resulting from the positive ion HPLC-MS data for the morning sample collection period is shown. One of the ions that contributed to this separation was m/z 206.0453, corresponding to C10H8NO4.When subjected to MS/MS analysis, this ion was provisionally identified, from the product ion spectra and elemental composition, as 4,8 dihydroxyquinoline-2-carboxylic acid,a metabolite found in the tryptophan pathway, and this was confirmed by comparison of the HPLC-MS/MS properties of an authentic standard. Similarly, female animals from the three strains were readily differentiated from each other by PCA, as indeed were male and female animals from all three strains [9].

Literature of review

Xiang Y, et al. (2006)

Fast liquid chromatographic (LC) methods are important for a variety of applications. Reducing the particle diameter (dp) is the most effective way to achieve fast separations while preserving high efficiency

R.S. Plumb, et al. (2002)

With this methodology it was possible to differentiate the control samples from the dosed samples and to identify the components of the mass spectrum responsible for the separation.

A. Lafaye, et al. (2003)

Use of reverse-phase liquid chromatography coupled to electrospray ion trap (QIT) mass spectrometry for the analysis of the metabolome chromatographic system is followed by a slow gradient elution and mass spectrometric detection in the scanning mode from m/z 100–1000 in both positive and negative modes.

E.M. Lenz, et al.(2004)

Demonstrate the complementary nature of the NMR and MS-based techniques for metabonomic analysis.

C.L. Gavaghan, et al. (2000)

Differences were observed in tricarboxylic acid cycle intermediates and methylamine pathway activity

Discussion

Interpretation of ICP-MS analysis to authentication and traceability would be easier in groups of food samples. Therefore, the products were divided into classes with particular features. Our aim is to provide and discuss the most important information for each group of products. Table 2 lists the published studies up to date (August 2022) referring to ICP-MS analysis as a method for geographical origin authentication of agricultural products and foods

Alcoholic Beverages

Wine belongs to the most strictly protected products worldwide. Fast and accurate analytical methodology for authenticity and traceability has become apparent. It has been shown that elemental analysis (trace and rare elements, and stable isotope ratios) is commonly used for the geographical origin of wines [14].

Other alcoholic beverages which have been studied by means of multi-element distribution are whiskey [15–16], cider [17,18], beer [19,20] and Chinese liquors [21]. Gajek and co-workers [16] studied extensively diverse varieties and ages of whiskey from different geographical regions and production procedures. Their investigations demonstrated that there were discrepancies in the metals Al, Cr, Cu, Fe, K, Mg, Mn, P, S, Ti, Tl, Zn, and V between single malt and blended Scotch whiskies. Furthermore, it was noteworthy that homemade whiskies from Poland are composed with the highest concentrations of Sr, K, S, and P. They were also observed that Cu, Mn, Zn, and P exhibit alterations in their concentrations during the aging of the samples. In case of beer, earlier this year, Lafontaineet al.

Dairy Products: Milk and Cheese

Trace element analysis in dairy products may derived from the metabolic pathwaysof the animals and the geographical regions of the farms. In 2008, Benincasa and co-workers [22] investigated the multi-elemental profile of 12 cow and 6 water buffalo milksamples. All the animals were treated equally in the same farm to identify elemental discrimination between the two animal species. Indeed, the authors achieved to differentiate the two types of milk based on their multi-elemental profile. Fernando's group [8]published, lately, a study which investigated the geographical origin authentication of cow milk from different territories in Sri Lanka by determining the stable isotope ratios of C,H, N, and O as well as the elemental composition with the IRMS and ICP-MS techniques,

respectively. It was proposed that a combination of stable isotope ratio of δ15N, δ13C (in milk casein), δ 15N, δ 18O, and δ 2H (in whole milk), and the concentration of the metals Li,Al, Cr, Mn, Fe, Co, Ba, and Sr could be promising markers to identify the geographical region of samples which were collected by four different agroclimatic zones. According to the authors the latter elements were possibly related to intrinsic factors of the animal (breed,lactation etc.), dietary habits and supplements. In similar studies, Tedesco et al. (2021) [23],and Aceto et al. (2017) [24] investigated the role of trace and rare earth elements in milk samples, throughout the production chain, from various regions of Italy. It was observed that the concentration of lanthanides remains nearly constant during the milk production and therefore were classified as suitable tracers.

Vegetables, Mushrooms and Fruits

Vegetables and fruits are products which are directly linked with the soil; thus, it couldbe an unambiguous correlation between their elemental fingerprints and the geologicalsetting of a region. Traceability and authenticity of vegetable and fruit foodstuffs can be a more straightforward procedure by comparing and relating the elemental distributions of samples and the soils. These days, the authentication of geographical origin of fruits and vegetables is a crucial requirement for food safety, due to the heavy demand for those products which has led to fraudulent labeling practices. Stable isotope analysis (C, N, H, O,S, and Sr) is considered as a pioneer in the field of food forensics. Multi-elemental analysis has been also used, in

combination with stable isotope ratio or by itself. Trace elements and lanthanides represent the mineralization characteristics of a particular region.Reviewing the literature, vegetables such as onion [25]

Conclusion

The analysis as a whole suggests that HPLC is a flexible, repeatable chromatographic method for the quantification of pharmacological products. In terms of quantitative and qualitative estimation of active compounds, it has numerous applications in numerous domains.

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