Food Analysis by HPLC

edited by
Leo M. L. Nollet

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Leo M. L. Nollet
Industriele Hogeschool van het Gemeenschapsonderwijs - C.T.L.

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Preface

High performance liquid chromatography (HPLC) is a fast technique that, with high precision and specificity, separates mixtures into individual ingredients. Used as a routine procedure, it has several advantages: it can be completely automated; sample cleanup and preparation are simple; and the reproducibility of the packing material means that the analytical conditions remain the same for a new column.

Different kinds of HPLC exist. Many kinds of column packings and solvents are available. Retention behavior and resolution are affected by column characteristics (C-loading, chain length, porosity, etc.) and by elution scheme characteristics (mobile phase, pH, organic modifier, etc.). The samples can be separated on the basis of solubility and polarity of the sample components.

For normal phase HPLC the solvent (mobile phase) is nonpolar (hexane, methylene chloride, etc.) and the column packing (stationary phase) is polar (silica). Sample molecules are more or less attracted to the particles in the column versus the solvent. The less polar molecules are first eluted. Reversed phase HPLC (RP HPLC) uses polar solvent (water, polar solvents or mixtures) and nonpolar column packing (C-18, phenyl). Here the elution order is reversed. The stationary phases are mostly derivatized with alkylsilane such as octadecyl groups (C-18 or ODS) or octyl-groups (C-8). In gel permeation chromatography (GPC) the separation of large molecules, such as proteins, is based on their effective size. Ion exchange chromatography separates samples based on their charge (anion or cation exchange). Tailor-made or specialty columns provide high-resolution separations of well-defined components such as fatty acids or hydrocarbons.

Applications of HPLC in food technology involve, on one hand, an analytical and qualitative testing of the product composition and, on the other hand, an assurance of product quality with an increase of productivity. HPLC is used in the food industry for the analysis of components in both raw and processed products. In a new food product, analysis of the raw materials, the intermediates, and the final products is necessary. Changes during processing or storage are to be followed as well. Foods and beverages may be tested on contaminants or additives, so that governmental regulations may be followed.

A large number of food components may be separated with HPLC. Although several books on HPLC separation methods of amino acids, vitamins, and carbohy-

drates have been published, there is no up-to-date, practical manual available. This book fulfills such a need, covering all major topics of food compounds for the food analyst or engineer. Chapters are written on amino acids, peptides, proteins, lipids, carbohydrates, vitamins, organic acids, organic bases, toxins, additives, antibacterials, pesticides residues, brewery products, nitrosamines, PAHs, and anions and cations.

The reader should find it helpful that the most recent published articles are discussed. Special attention is paid to reversed phase separations without neglecting the other mentioned HPLC techniques. Specialists describe in detail sample preparation and separation conditions. The applications to food chemistry are directly usable.

This book will find a large audience in the fields of chromatography, analytical chemistry, and, especially, food chemistry and food technology.

Sincere thanks are given to everyone who has contributed. I would like to thank all contributors for their excellent efforts.

Leo M. L. Nollet

Contributors

Leon Baert Physical and Radiobiological Chemistry, University of Ghent, Ghent, Belgium

Jean-René Bailly Microbiologie Appliquée, Université de Perpignan, Perpignan, France

G. F. M. Ball Scientific Services Division, J. Sainsbury, plc, London, United Kingdom

D. Blanco Gomis Departamento de Quimica Física y Analítica, Universidad de Oviedo, Oviedo, Spain

Nickos A. Botsoglou Department of Animal Production, Ichthyology, Ecology and Protection of Environment, Aristotelian University School of Veterinary Medicine, Thessaloniki, Greece

Rodney J. Bushway Department of Food Science, University of Maine, Orono, Maine

John R. Byrne Analytical Section, Guinness Brewing Worldwide Research Centre, Dublin, Ireland

Neil D. Danielson Department of Chemistry, Miami University, Oxford, Ohio

Greet Debruyckere Laboratory of Food Analysis, University of Ghent, Ghent, Belgium

André De Leenheer Laboratory Medical Biochemistry and Clinical Analysis, University of Ghent, Ghent, Belgium

Georges de Saint Blanquat Department of Agroresources, University of Bordeaux 1, Foulayronnes, France

Walter Flak Bundesanstalt für Weinbau, Eisenstadt, Austria

Dolores González de Llano Instituto de Fermentaciones Industriales, Consejo Superior de Investigaciones Científicas, Madrid, Spain

R. J. Hart Biopolymers Section, Leatherhead Food Research Association, Leatherhead, Surrey, United Kingdom

A. Huyghebaert Laboratory of Food Technology, Chemistry and Microbiology, University of Ghent, Ghent, Belgium

Dimitrios C. Kufidis Department of Animal Production, Ichthyology, Ecology and Protection of Environment, Aristotelian University School of Veterinary Medicine, Thessaloniki, Greece

Willy E. Lambert Laboratory Medical Biochemistry and Clinical Analysis, University of Ghent, Ghent, Belgium

Jacques Leitao ONET Agro Alimentaire, Z. A. Mane Coëtdigo, Vannes, France

Domenico Marini Ministero delle Finanze, Laboratorio Chimico Centrale Dogane, Rome, Italy

Ian McMurrough Guinness Brewing Worldwide Research Centre, Dublin, Ireland

Leo M. L. Nollet Department of Biotechnology, Industriele Hogeschool van het Gemeenschapsonderwijs-C.T.L., Ghent, Belgium

Leopold Pilsbacher Bundesanstalt für Weinbau, Eisenstadt, Austria

M. Carmen Polo Instituto de Fermentaciones Industriales, Consejo Superior de Investigaciones Científicas, Madrid, Spain

Mercedes Ramos Instituto de Fermentaciones Industriales, Consejo Superior de Investigaciones Científicas, Madrid, Spain

Fraser W. Scott Nutrition Research Division, Food Directorate, Health and Welfare Canada, Ottawa, Ontario, Canada

Toshihiko Seki Sendai Municipal Institute of Public Health, Sendai, Japan

N. P. Sen Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada

Jeffrey H. Sherman* Department of Chemistry, Miami University, Oxford, Ohio

* *Current affiliation: Clark Processing, Inc., Dayton, Ohio*

A. M. Siouffi Laboratoire de Génie Chimique et Chimie Appliquée, Université d'Aix-Marseille 3, Marseille, France

Katsumi Tamakawa Division of Food and Environmental Hygiene, Sendai Municipal Institute of Public Health, Sendai, Japan

Akira Tsunoda Sendai Municipal Institute of Public Health, Sendai, Japan

Jan Vanderdeelen Department of Physical and Radiobiological Chemistry, University of Ghent, Ghent, Belgium

Paul Van der Meeren Belgian National Fund for Scientific Research, Department of Physical and Radiobiological Chemistry, University of Ghent, Ghent, Belgium

Carlos Van Peteghem Laboratory of Food Analysis, University of Ghent, Ghent, Belgium

Jan Vervaeck Laboratory of Food Technology, Chemistry and Microbiology, Faculty of Agricultural Sciences, University of Ghent, Ghent, Belgium

Judy A. White Biopolymers Section, Leatherhead Food Research Association, Leatherhead, Surrey, United Kingdom

Contents

1
HPLC

A. M. Siouffi *Université d'Aix-Marseille 3*
Marseille, France

INTRODUCTION

Since its advent in the early 1970s high performance liquid chromatography (HPLC) has evolved toward a high degree of sophistication. At the present time liquid column chromatography (LC) is utilized to consider a fully instrumentalized system. LC has become a routine method in almost all areas of instrumental analysis, and its continuous growth seems assured. Fully automated systems are available at reasonable prices, and the presence of the analyst in the close vicinity of the instrument is no longer required. Very efficient columns are capable of supplying thousands of plates per meter, and manufacturers have directed a great deal of effort towards reproducibility.

Basically the LC instrument is made of a solvent reservoir, a pump, an injection device, a column, a detector, and a data acquisition system (Fig. 1). The column is the heart of the system, and two solutes cannot be electronically resolved. Much effort has been paid to the instrument as it was true some years ago that excellent separations achieved in the column could be ruined by a poorly designed instrument. Hyphenating LC separation with spectroscopic identification has been greatly developed and LC/MS, LC/FTIR are readily available. Analytical purposes represent the vast majority of applications, but preparative scale is becoming increasingly accepted as a production tool.

The volume of literature on LC is so large that complete coverage is impossible. A survey is published biannually by *Analytical Chemistry* and does not include applications, which may be retrieved from the bibliography section of the *Journal of Chromatography.* Fortunately, computerized retrieval systems are now readily accessible. Table 1 (which is not exhaustive) shows some advantages and drawbacks of the technique.

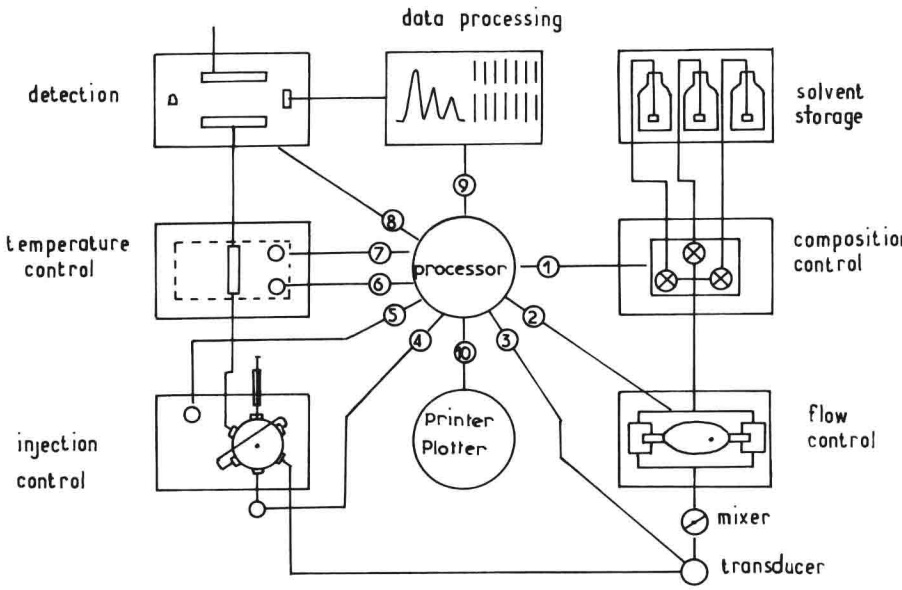

Figure 1 A scheme of a typical LC instrument. (Courtesy of Hewlett-Packard.)

Table 1 LC Features

Advantages	Drawbacks
Very high variety in mobile phase selection	Available stationary phases less numerous than in GC
Very high variety in retention mechanism	Toxicity, high viscosity, miscibility of solvents
No destructive detectors	Lack of a very quasi universal detector like FID in GC
Direct injection of some complex mixtures	Deterioration of the instrument with corrosion
Possible transfer of data from TLC	Hyphenating with MS more tedious than with GC
Fast analysis	Instrument more expensive and more difficult to use than GC
Possible connection of very efficient columns	

TRENDS

Fundamental advances in LC are occurring in the areas of column packings and detection technology. Miniaturization of the separation system will be complemented by automation of the sample treatment.

New efficient and selective phases will certainly appear, and modifying in situ the nature of the ligand would be highly valuable. It is now fully recognized that reverse phase columns are not capable to separate any kind of solutes. The situation is similar to that observed in the early 1980s when bare silica was utilized almost

exclusively. More and more specialty columns will appear. Column design to day has reached a very advanced stage of development.

According to Scott (1), analysis times may be reduced to a few milliseconds for the separation of solutes having relatively large separation ratios by employing small columns packed with the smallest available particles and operated at very high mobile phase velocities. Open tubular columns are still in the domain of research laboratories and are not in current practice for routine work. However, these columns opened up a number of possibilities, and the analysis of single cells has recently been achieved (2). Detection of ventomoles of solutes have been reported (3). It opens a new era which will involve an actual challenge in the purity of solvents. Computer-assisted optimization methods are more and more popular. The topic is now well documented in many books and monographs (4,5). Personal computers have changed the laboratory procedures and excellent softwares are now appearing. Nevertheless, one must be aware that computers are an aid to the chemist and do not think in the place of the chemist.

SOME USEFUL DEFINITIONS—CRITERIA FOR COLUMN PERFORMANCES

Retention and Selectivity

In a chromatographic system the solute is distributed between the stationary and the mobile phases. When a solute exhibits no affinity for the stationary phase, it travels through the column at the velocity of the liquid. It is eluted at the time t_o, which is the retention time of the unretained solute

When the column is packed with porous particles the velocity u of the mobile phase is given by the Darcy equation:

$$u = \frac{Bo\ d_p^2 \Delta P}{\eta L}$$

where Bo is the Karman Kozeny permeability, d_p is the particle mean diameter, ΔP is the pressure drop, η is the viscosity of the solvent, and L is the length of the column.

$$Bo = \frac{\epsilon^3}{150\ (1 - \epsilon)^2}$$

ϵ is the porosity and may be divided into two parts: ϵ_i, the intraparticular porosity corresponding to the liquid entering the pores, and ϵ_e, the extraparticular one corresponding to the liquid that is external to the particles.

As a consequence the velocity of the liquid is much higher in the extraparticular volume. This difference in velocity is the basis of size exclusion chromatography. We must distinguish the "unretained" solute from the "excluded" solute: the latter never enters the pores. Accurate determination of t_o is thus tedious and rather fallacious as it depends on the type of solute selected; every molecule may have its own void volume. A discussion of the problem can be found in Refs. 6–8. From a practical point of view the method for determination of t_o should be mentioned in an experimental section. We can write $t_o = L/u$. When a solute