FOOD QUALITY CONTROL BY HYPHENATED SEPARATION TECHNIQUES

Justyna Walczak[1,2], Paweł Pomastowski[1,2], Bogusław Buszewski[1,2]

[1]Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University
[2]Interdisciplinary Centre of Modern Technology, Nicolaus Copernicus University

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Summary: Food as complex mixture of proteins, lipids, vitamins, etc. cannot be separated and identified by using in only one method. This article presents a revision on the hyphenated chromatographic techniques and methods used in food analysis and described main application in food science research, and determination of xenobiotics and their metabolites in environmental. Also article discusses applications of "omics" in food analysis (proteomics, transcriptomics, genomics, metabolomis) and new discipline of - foodomics.

Keywords: food analysis, hyphenated techniques, liquid chromatography, mass spectrometry

Introduction

Food analysis is located within the scope of multidisciplinary food science and human nutrition, which used the achievements in areas, such as biology, chemistry, microbiology or mathematic. The main objective is to evaluate the quality of food, which is primarily determined by the chemical composition. In order to provide high quality of healthy food, all parts of the food chain (production, sourcing of raw material, purchase of raw material, processing and manufacturing), must be subject to strict supervision designed to find the factor which reduce the quality of healthy food and provide appropriate food safety. Knowledge of the methods for the determination of basic food ingredients, food additives, contaminants or changes during processing and storage of food is essential for specialists involved in the production and quality control of food. It is also useful for all people interested in understanding the relationship between food consumed and human health (Mcgorrin 2009).

Food analysis is a very hard task, due to the complexity of the matrix which is typical of food. In order to characterize and identify specific food components, hyphenated separation techniques are used. Such hyphenated techniques are of use in food analysis, pharmaceutical industry or medicine. Analytical techniques have been classified according to the methods of sample preparation (e.g., solid phase extraction (SPE); liquid-liquid extraction (LLE); purge and trap (PT); polymerase chain reaction (PCR)), separation techniques (e.g., high performance liquid chromatography (HPLC); gas chromatography (GC), capillary electrophoresis (CE); thin layer chromatography (TLC); two dimensional gel electrophoresis (2DE)), and identification methods (e.g., ultraviolet (UV); mass spectrometry (MS); matrix-assisted laser desorption/ionization (MALDI); nuclear magnetic resonance (NMR)) (Michel, Buszewski 2008).

This paper describes the most important applications of the hyphenated separation techniques in food science and technology. It describes analysis of food-related molecules, such as amino acids, peptides, proteins, lipids, vitamins, metabolites, toxins, pesticides, and antibiotics. Moreover, among environmental pollutants there can be found large group of xenobiotics. Xenobiotic is a natural or synthetic chemical compound which is not a product of biosynthesis. Compared to the naturally occurring organic substances, some xenobiotics and their metabolites are resistant to biodegradation.

Address for correspondence: Bogusław Buszewski, Nicolaus Copernicus University, Gagarin 7 Street, 87-100 Torun, e-mail: bbusz@chem.umk.pl, phone: +48 56 611 43 08

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Food analysis

Food quality is an important factor in determining the state of human health. The presence of toxic and harmful to health substances in food is an important issue and needs to project both remedies for the prevention of pollution, as well as methods of control to assess the degree of contamination of the final product. A particular group of food contaminants are pesticides. These substances in small amounts can cause acute poisoning, contribute to the formation of cancer and developmental defects, adversely affect the endocrine, immune and nervous systems. Analysis of food samples for the presence of pesticides is a multi-stage analytical procedure including collection and sample preparation in this extraction of analytes from the matrix, the purification of extracts and determination of pesticides (Kumar et al. 2010). Proper extraction of the sample and its proper preparation are crucial because they determine the quality and credibility of the result of the determination. Over the last decade there has been a significant growth and development of the extraction methods used for the isolation of pesticides from food, which has allowed to simultaneous extraction of the remains of many substances with different physicochemical properties. The new methodology for determining pesticides residues in food matrices is so-called QuEChERS (quick, easy, cheap, effective, rugged, and safety) - solvent extraction combined with purification of the extract (Payá et al. 2007). Liquid chromatography coupled with mass spectrometry (LC-MS) is currently one of the most common methods used for detection, identification and quantification of pesticides in food. This technology provides information about the structure of the analyte which does not demand thorough purification of a sample, and can do without derivatization of the analyzed compounds (Lehotay 2007).

Another very important issues is analyzing antibiotics residue in food samples (Moreno-Bondi et al. 2009). A wide application of antibiotics in animals husbandry makes it necessary to conduct the quantitative and qualitative control of residues of these medicinal substances in food. Antibiotics are also added to food or water, because due to this a positive effect on weight gain of animals can be observed. However, the antibiotic remaining in the tissues and organs of animals and their products - milk, eggs and honey are potential source of adverse effects on consumers of food of animal origin. Small doses of antibiotics taken with food for a longer period, may contribute to the emergence in human body of drug-resistant bacterial strains. Taken with food, even small amounts of antibiotics may cause allergic reactions or disturbances in the normal functioning of tissues or organs (mutagenic and carcinogenic effects) (Pejsak, Truszczyński 2005). The most popular extraction technique for determination antibiotics in food is solid phase microextraction (SPME). A great advantage of applying this technique is the small sample volume and low solvent consumption. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is most often useful technique in this case (Szultka et al. 2014). Figure 1 presents the application of SPME coupled with HPLC-MS in determination of amoxicillin. The use of this technique allows efficient detection and confirmation of the presence of antibiotics and other antibacterial drugs in the products and foodstuffs of animal origin (Buszewski et al. 2011).

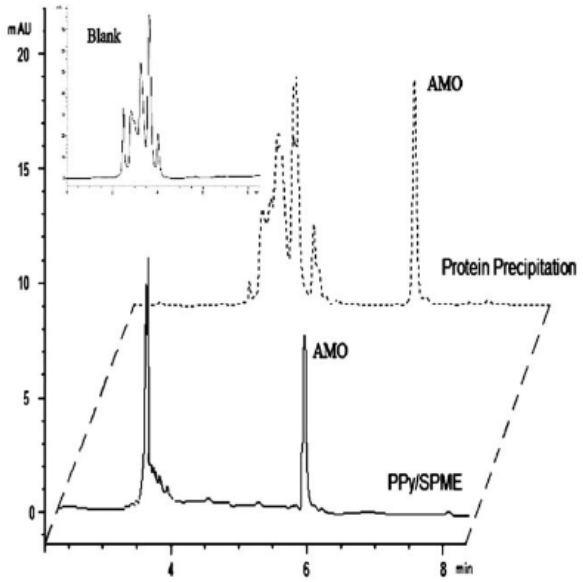

Figure 1. Chromatogram of amoxicilin extracted by PPy/SPME and protein precipitation (according to Buszewski et al. 2011)

High performance liquid chromatography (HPLC) or ultrahigh performance liquid chromatography (UHPLC) are the most frequently used techniques in analyzing food components. Gas chromatography is often used in an analysis of volatile compounds, such as fatty acids (Golay 2009). Liquid chromatography coupled with mass spectrometry

(MS) or tandem MS (LC-MS/MS) have been applied particularly to analyze antimicrobial residues in food of animal origin (Bogialli, Di Corcia 2009), and food allergen (Faeste et al. 2011). The essential oils (Jalali-Heravi, Parastar 2011) and food contaminations (Robledo, Smyth 2009) can be identified by using other hyphenated separation techniques: gas chromatography with mass spectrometry (GC-MS) or capillary electrophoresis-mass spectrometry (CE-MS).

Multidimensional chromatography techniques in *off-line* and *on-line* mode, such as LC×LC, GC×GC, LC×GC, are very useful techniques in analysis and separation of complex mixtures (Herrero et al. 2009, Welke, Zini 2011). Differences in columns properties (e.g. HPLC x HPLC system) change the selectivity and resolution of separation system drastically. In results of set-up combination (see Fig 2) in the first cycle (column No. 1) fraction from separated mixture (chromatogram) can be recived, but in the second cycle (column No. 2) the interesting analytes have been obtained in pure form.

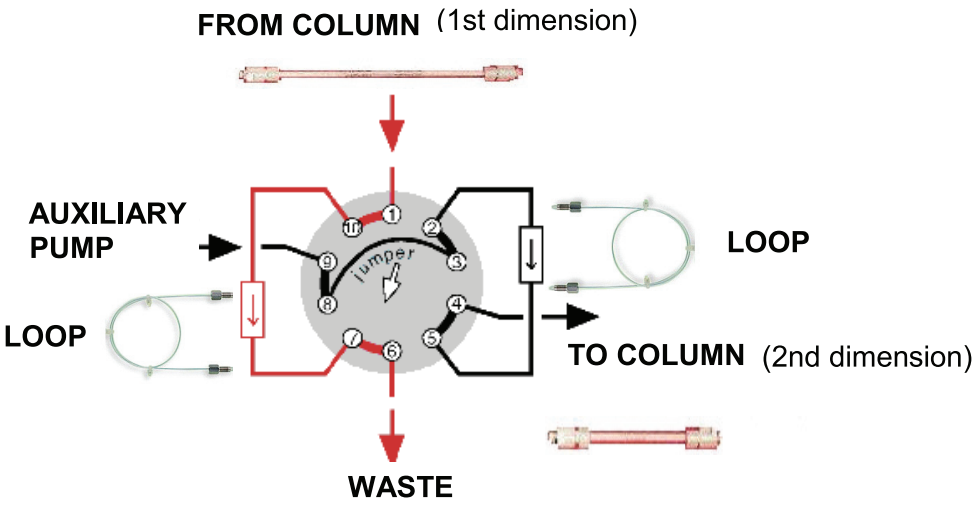

Figure 2. Two dimentional LC x LC set-up

The combination of two systems can help to identify and separate phospholipids in egg yolk (Walczak et al. 2014). The Figure 3 show the chromatogram of phospholipids from egg yolk. Thirteen fractions of PLs obtained from the first dimension have been analyzed by RP-HPLC (Fig 3a) coupled with mass spectrometry. N,O-dialkylphosphoramidate (C18) (Fig 3b) stationary phase was used. In the first dimension separation is based on hydrophobicity of fatty acids/or hydrophobic interactions with stationary phase (differences in chain lengths and number of double bonds of acyl residues). In the second dimension (with the retention time on Fig. 3b) separation is based on the differences that take place in the polarity of the phospholipid "headgroups" take place.

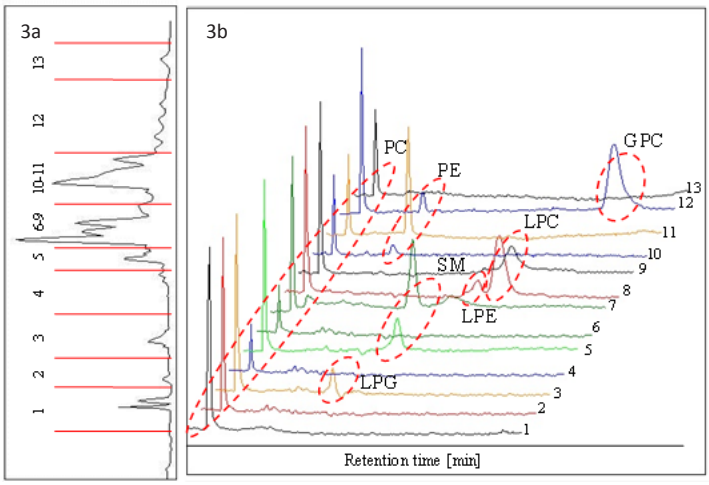

Figure 3. Chromatogram of egg yolk phospholipids separation: a) first dimension; C18 column, mobile phase: 100% MeOH, flow 1 mL/min, b) second dimension; *N,O*-dialkylphosphoramidate (C18), mobile phase: 90% MeOH/10% H_2O, flow 0.45 mL/min. The number of collected fractions in the chromatogram **3a** corresponds to the number of chromatograms in the **3b** (according to Walczak et al. 2014)

Foodomics as a new approach in food analysis

In recent decades, "bioanalytical chemistry" has become a very important and a rapidly developing branch of chemistry. The concept of bioanalytical chemistry comprises two aspects. In first meaning it refers to studies whose target are objects and/or biological phenomena. In the second case, bioanalytical chemistry is the study of the use of "bio-tools" whose devices are integrated with biomolecules. Figure 4 shows the relationship between bioanalytics and "omics", and other specialty areas such as drug metabolism and pharmacokinetics, clinical chemistry, toxicology and safety pharmacology.

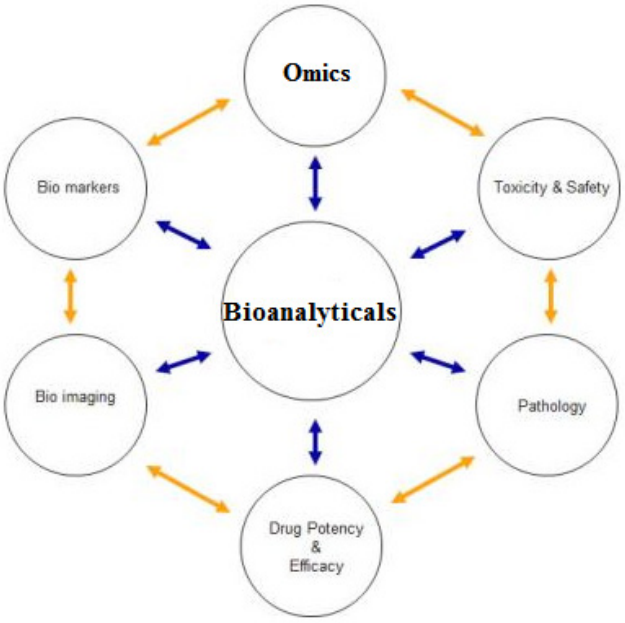

Figure 4. Relationship between bioanalyticals and specialty areas

Foodomics is a new multidiscipline in "***omics***" technologies. We can define foodomics "as a discipline that studies the food and nutrition domains through the application of advanced omics technologies to improve consumer's wellbeing, health, and knowledge" (Cifuentes 2009). Foodomics include transcriptomics, genomics, proteomics, and metabolomics, and a variety of ***omics*** sub-disciplines (epigenomics, lipidomics, metallomics, diseasomics, etc.). Genomics analysis investigates expression of genes, designation of RNA in a biological sample and the indication of genes mutations. Polymerase chain reaction (PCR) analysis faciliates forecasting and studying neoplastic diseases. The main purpose of proteomics is the detection, quantitative determination of protein and identification of biomarkers in the early stages of the disease. Proteomic studies use chromatographic and electrophoretic separation, mass spectrometry, immune reactions, protein and tissue microarray. Metabolomics studies are very complicated by the presence of hundreds or thousands of chemically labile metabolite present in the sample. Lipidomics as a new branch of molecular biology deals with the characterization of lipids present in living organisms, their interactions, and biological functions. Genomics with proteomics, metabolomics and transcriptomics are the main backbone of ***foodomics*** (Figure 5).

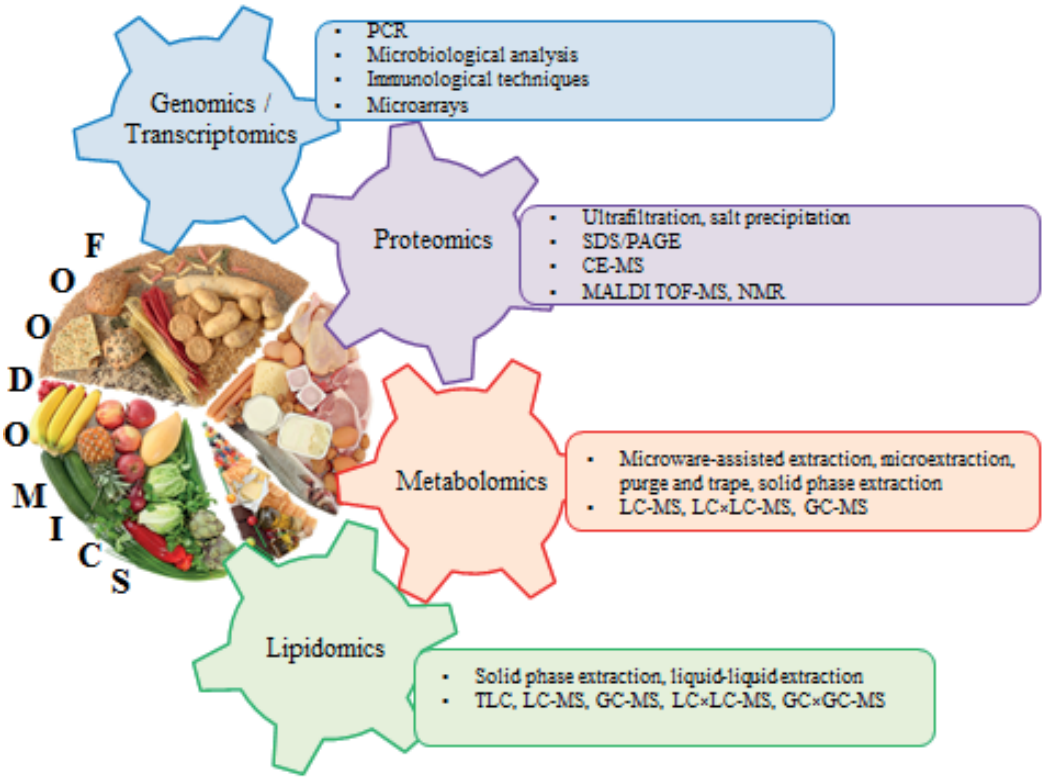

Figure 5. Scheme of foodomics platform, including analytical methodologies

Conclusion

Over the last two decades the hyphenated separation techniques have significantly broadened their applications in many areas of science, industry, pharmacy. In the paper various hyphenated methods, e.g., LC-MS, GC-MS, CE-MS, etc. have been presented which are used in such processes as pre-isolation analyses of crude extract/fraction, isolation, detection, and identification of products.

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