separations

Editorial

Special Issue: Analysis of the Main Classes of Lipid (Fat and Oil) Components in Food and Blood by Using HPLC and Gas Chromatographic Techniques

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In many cases in science, many discoveries are made by pure chance, as happened for example to Alexander Fleming (Darvel (Scotland) 6 August 1881–London (England) 11 March 1955) who, while observing slides under a microscope in 1922, a few weeks after putting his nasal mucus on a Petri dish, noticed that cultures of microbes had developed all over the plate, except for his secretion. Subsequent experiments, performed with other mucus or with tears, showed him that an antibacterial substance was present in these liquids. From these casual intuitions, he opened the doors to the discovery of penicillin, the first substance with an antibiotic effect [1,2]. From this discovery, which only occurred a century ago, many antibiotic compounds were isolated from microorganisms, and many others were synthesized by organic and pharmaceutic chemists [3–5].

The same can be said of Michail Semënovič Cvet (also transcribed as Tswett, he was born in Asti (Italy), 14 May 1872, died in Voronezh (Russia), 26 June 1919) who discovered chromatography as a phenomenon by pure chance in 1901, during his research on plant pigments [6–9].

He used a "chromatographic column" for liquid–solid absorption, with calcium carbonate as the adsorbent and mixtures of petroleum ether and ethanol as eluents to separate the chlorophyll and carotenoids. This intuition was based on the fact that chlorophyll and carotenoids are not polar substances, and so they cannot be strongly retained by "stationary phase" constituted by calcium carbonate. Tswett was lucky in this case because he observed the formation of different colors (bands) through the column as the organic mixture of liquids leached on it, and so he named this phenomenon "chromatography"—from Greek, meaning the "envelope of color". Starting from pigment color extract (green-brown), he observed different pure colors forming through the column (green, yellow, orange). Two unfortunate possibilities could have been true: (1) the sample placed at the top of the column could have eluted with the solvent and, therefore, no colors would have been seen to form (no retention by the solid phase); (2) the sample placed at the top of the column could have been irreversibly retained by the solid phase—likewise, in this case, no colors would have been seen along the column (very strong retention by solid phase). These two extreme cases indicate that chromatographic conditions are not good for specific samples, and they have to be avoided for chromatography to be successful. The method was presented on 30 December 1901 at the XI Congress of Naturalists and Physicists (XI съезд естествоиспытателей и врачей) in Petersburg [7]. The first printed description came out in 1903, in the proceedings of the Warsaw Society of Naturalists, biology section. He first used the term "chromatography" in 1906 in his two articles on chlorophyll in the German botanical journal *Berichte der Deutschen botanischen Gesellschaft* [8]. In 1907, he demonstrated his method to the German Botanical Society [9].

This simple experiment opened the doors to chromatography, but the right interpretation of the phenomenon came about later. As is more usual in science, a new discovery is not always immediately accepted by the scientific community, and so chromatography

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remained in standby for about forty years since Archer John Porter Martin (1 March 1910, London (England)–28 July 2002 Llangarron (Wales)) and Richard Laurence Millington Synge (Liverpool (England), 28 October 1914–Norwich (England), 18 August 1994) developed partition chromatography to separate the chemicals with only slight differences in partition coefficients between two liquid solvents; this further and new application of chromatography earned the two researchers the Nobel Prize in Chemistry in 1952. However, Michail Cvet is rightly remembered as the inventor of chromatography, of which he laid the foundations (even though, not completely! However, it does not matter!) in 1901 during his research on plant pigments [6].

The power of chromatography comes from its ability to separate a mixture of compounds, or "analytes", and determine their respective identity (chemical structure) and concentration. For this reason, all chromatographic techniques work at qualitative and quantitative levels. The term "chromatography" is nowadays extended to all molecules, even if they are not colored to human eye, and so the universal significance of the term is "separation". Chromatography can be divided into three basic types, which includes gas, liquid and supercritical fluid chromatography. These techniques are different for the mobile phase: gas–solid and gas–liquid chromatography (gas chromatography (GC)); liquid–liquid and liquid–solid chromatography (chromatography (LC)); supercritical fluid–solid and supercritical fluid–liquid chromatography (supercritical chromatography (SFC)). Liquid chromatography can be further divided into ion exchange, separations based on size (exclusion chromatography (GPC)) and even extended to gel-based electrophoretic techniques, which are techniques of separation based on the application of an electric field, so that the molecules are separated on the basis of mass-charge ratio.

In general, each type of chromatography comprises two distinct steps: chromatography (or separation of individual compounds in distinct elution bands) thanks to specific stationary phase immobilized under the form of a column and identification based on detection of each elution band. Briefly, gas chromatography is the process of taking a sample (liquid or gaseous) and injecting it into the instrument, called gas chromatograph, turning the solvent and analytes into gaseous form (for liquid samples) and separating the mixture of compounds into individual peaks, each of which can be linked to individual compounds. Liquid chromatography completes the same process, except the separations occur in a liquid phase. Individual band or peaks exit the column, and identification occurs by a specific detector (ECD: electron capture detector; NPD: nitrogen phosphorous detector) or a relatively universal detector (TCD: thermo-conductivity detector; FID: flame ionization detector). One particularly common and universal detector for both gas and liquid chromatography is mass spectrometry detector (MSD), which transforms each analyte, after the elution from column, from a chemically neutral species into a positive cation, usually breaking various bonds in the process. Detecting the mass of individual pieces (referred to as fragments) allows for conclusive identification of the chemical structure of the analyte; for this reason MSD is nowadays considered the most powerful detector for chromatography.

From this brief excursus of state-of-the-art chromatographic techniques, it is clear that science has made great strides in the last fifty years thanks to the improvement of chromatographic techniques. The first approaches to specific separations were in the field of foods (analysis of fats and oils), especially for separations of fatty acid methyl esters, which can be assumed as the first complex separation, because fatty acids in fats and oils start from butyric acid (C4) (butteroil) and arrive at lignoceric acid (C24) (fats and oils), and so the first packed columns were capable of eluting compounds till 240 °C [10].

From the first separations on packed columns, step-by-step separations were moved on capillary columns at the beginning of the 1990s; this new technology of constructing columns gave a new impulse to gas chromatography because, while the packed columns had to be assembled by the operator, the new capillary columns were being sold (and are presently sold) ready to use, and so the analyst can work more rapidly than in the case of a packed column that required long stabilization time. In addition, capillary columns

result in higher efficiency and resolution of separations in respect of the old packed type. A contribution to the improvement of analysis methods of different classes of lipids was given by my research group in the last twenty years. In the food sector, for quality control, fraud detection and authentication of butter fat and other oils and fats, three methods have been developed: one for triglyceride analysis and two for fatty acids analysis. The various analytical methods, based on high-resolution chromatographic techniques, have been applied to the study of the composition of different food matrices with one composition mainly consisting of triglycerides (butter, olive oil, lard) [11]. For the determination of the composition of fatty acids in food matrices with a composition mainly consisting of triglycerides, two innovative methods of transesterification of triglycerides in the pentyl and phenethyl esters of fatty acids were developed. Conversion to pentyl esters of fatty acids avoids the loss of butyric acid compared to when sampling fatty acids are derivatized as methyl esters of fatty acids. Finally, in the HPLC analysis, the conversion of fatty acids in the phenethyl esters allowed for a separation of the unsaturated fatty acids without degradation compared to GC analysis. Additionally, using the same capillary column (RTX 65-TG) with 65% phenyl-35% methyl silicon stationary phase, a method is presented based on gas chromatographic analyses for the quantification of cholesterol content in eggs [12] and the determination of cholesterol content in egg pasta to establish the total number of eggs added to semolina [13]. Wax esters and steryl esters were determined in olive oil; this procedure was proposed as an alternative method for the procedure proposed by the EEC Regulation 183/93. By using the capillary column RTX 65-TG (Restek), separation of analytes was deeply improved in respect of separation obtained by using capillary colum with 5% phenyl methylsilicone stationary phase [14]. Lastly, the minor lipid component contained in other types of food, such as flour and semolina, is also analyzed. These foods were analyzed for wax and steryl esters [15].

The aim of Special Issue "Analysis of the Main Classes of Lipid (Fat and Oil) Components in Food and Blood by Using HPLC and Gas Chromatographic Techniques" is to focus the attention of researchers in the fields of food and medicine on studying the composition of fats, oils and lipids contained in foods before the assumption and transformation that they undergo after the assimilation by human, in order to establish which types of lipids are transported in blood and what kind of analytes will be found depending on time after assumption. These studies are very important to better understand the metabolism of the main classes of lipids contained in foods (triglycerides, fatty acids, sterols, steryl esters and wax esters). It is worth noting that the same chromatographic method could be applied to the analysis of lipid components both in food and in blood after specific sample preparation; at the end of this procedure, a liquid sample, containing lipid components (triglycerides, fatty acids, sterols, steryl esters and wax esters) will be analyzed in the same chromatographic conditions by using high-performance liquid chromatography (HPLC) and/or gas chromatography on capillary columns.

Chromatography is nowadays the most powerful instrumental and analytical technique that can be applied to separation of complex mixtures of organic compounds in environmental, food, medicine and chemistry sectors.

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