



A REVIEW ON THIN LAYER CHROMATOGRAPHY METHODS FOR DETERMINATION OF AUTHENTICITY OF FOODS AND DIETARY SUPPLEMENTS

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ABSTRACT

This paper reviews advances in qualitative and quantitative thin layer chromatography analytical methods for assessing quality and authenticity of foods, beverages, and dietary supplements. This method also includes use of thin-layer chromatography in the assessment of the quality of lutein-containing dietary supplements. The new applications of various chromatographic techniques such as gas-liquid chromatography, high-performance liquid chromatography and electrophoretic methods employed for the analysis in macro- and micro-components in vegetable oils and dairy products are compiled and critically evaluated. The employment of these methods for authenticity tests and traceability is discussed.

KEYWORDS: Chromatography; Vegetable Oils; Dairy Products; Authenticity Test; Traceability; Lutein Containing Dietary Supplements.

INTRODUCTION

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Chromatography was discovered by M. Tswett in 1906. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide, or cellulose (blotter paper). This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved. Thin layer chromatography can be used to: Monitor the progress of a reaction, identify compounds present in a given substance, and determine the purity of a substance. Separation of compounds is based on the competition of the solute and the mobile phase for binding places on the stationary phase. For instance, if normal phase silica gel is used as the stationary phase it can be considered polar. Given two compounds which differ in polarity, the more polar compound has a stronger interaction with the silica and is therefore more capable to displace the mobile phase from the binding places. Adulteration is generally motivated by maximizing profit by adding a cheaper ingredient. The growing interest in the authenticity of foods and food products requires reliable verification methods because the properties of foods from different origins can also be different. Furthermore, traceability is

required more and more by consumers and government organizations because it is a significant component of overall food safety (Anklam and Battaglia, 2001). The methods used for the verification of food authenticity and traceability have to be suitable for the prevention of deliberate or accidental mislabeling. They are important for commercial reasons and play a considerable role in the assurance of public health. Moreover, these methods have to be able to link a finished product to its ingredients. As the origin is a decisive characteristic of foods, rapid, inexpensive and efficient analytical methods are demanded for the measurement of this criterion. These analytical techniques have to be applied in food manufacturing industry for process performance evaluation, for the detection of faults and to achieve a continually high-quality food product. A considerable number of methods have been developed for the assessment of the authenticity and traceability of foods. These include the successful application of polymerase chain reaction (Ahmed, 2002), various DNA methods (Hold et al., 2001), enzyme immunoassays (Roux et al., 2000), nuclear magnetic resonance spectroscopy (Gil et al., 2000), infrared and near-infrared spectroscopy combined with principal component analysis (PCA; Schulz et al., 2003), mid-infrared and Raman spectroscopies (Marigheto et al., 1998) and headspace-mass spectrometry (Lorenzo et al., 2002). Various chromatographic techniques offer a unique possibility for the rapid and reliable separation and quantitative

determination of macro- and microcomponents of highly similar chemical structures in complicated matrices of foods and food products (Forgács and Cserhádi, 2003; Nollet, 2003). Because of their advantageous separation characteristics, numerous chromatographic techniques have been tested, accepted and employed in the analysis of food and food products. Chromatographic methods can be equally employed for the measurement of the component of foods, the amount of legal or illegal additives, environmental pollutants and xenobiotic those are health hazards. Because of the high separation capacity of chromatographic techniques, they can be used for authenticity and traceability tests of foods. The aim of this review is the collection and concise enumeration of the most decisive new results in the field of the employment of various chromatographic techniques suitable for the authenticity and traceability tests of foods and the critical discussion of the results.

Thin layer chromatography first described in 1938 is similar to paper chromatography. However, instead of using paper as stationary phase, it involves adsorbent materials like silica gel, inert thin layer substrate. Before 1960 workers prepare their own plates in the laboratory but the procedures were often messy, arduous and resulted in many rejects. After this period, the workers only pre-prepare their own plates if they require a special type of layer that is not commercially available, because several convenient precoated plates are commercially available.^[2] Currently, beyond different types of TLC plates, there are kits ready to extraction and elution (A400A19, A400B11), including Solid Phase Extraction Vacuum System (A580), standards (discs with impregnated drugs, e.g. Morphine, codeine, meperidine, methadone, and propoxyphene—AN121), and chromatograms for separation of acidic, basic and neutral drugs which are sets TOX-LAB by Varian. When compared to PC, the TLC method has the advantages of faster runs and better separations, being more sensitive, more reproducible and allowing the choice among different adsorbents. Another advantage of TLC is the resolution, which is greater than in PC because the particles on the plate are smaller and more regular than paper fibers. Therefore, TLC has largely replaced paper chromatography.^[2,17] Developed for semi quantification of biogenic amines in fish.^[7] TLC is a popular chromatographic technique widely used for a variety of applications including analysis and purification, usually employed in the laboratory. Although the chromatographic resolution of TLC is typically lower than the resolution of HPLC, a number of advantages still make TLC an indispensable analytical tool.^[10] TLC is used to separate non-volatile mixtures.^[17] and is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide or cellulose. This layer of adsorbent is known as the stationary phase. The samples and standards must be applied on the plate which is then placed in a chromatography recipient containing a solvent or solvent mixture (known as the

mobile phase) which is drawn up the plate via capillary action. The different analytes ascend the TLC plate at different rates and the separation is achieved. In other words, like all other chromatographic methods, the separation process of complex mixtures by TLC is based on the different affinity of the analyte with the mobile and stationary phases. In relation to the adsorbent material used to make the plates, silica gel is the most widely used adsorbent being employed on the separation of several compounds in several food matrices, including products of animal origin by using the adsorption mechanism. However, other adsorbents may be used such as alumina, cellulose, polyamide and others. When compared to alumina, one of the major advantages of silica gel is that it has little or no tendency to catalyze unwanted degradation.^[18] Usually, aluminum oxide plates are used for the determination of basic substances, alkaloids, steroids; microcrystalline cellulose for the detection of amino acids, sugars and antibiotics; and RP-2, RP-8 and RP-18 (silica gel plates chemically modified with alkyl groups) to analyze fatty acids, carotenoids, steroids, and cholesterol and its esters.^[19]

TLC Methods in Foods

TLC can be used to monitor the progress of a reaction, to identify, semi-quantify or quantify compounds present in a given mixture as well as determine the purity of a substance.^[6] TLC is based on the differential migration of the components of a mixture which occurs due to different interactions between the mobile phase and the stationary phase. The wide variety of combinations among mobile and stationary phases turns this technique extremely versatile and widely applicable.^[13] in environmental, clinical, forensic, pharmaceutical, food, flavors, and cosmetics fields. Within the foods of animal origin, TLC may be applied to determine a variety of compounds such as lipids, vitamins, carbohydrates, amino acids, organic acids, proteins, enzymes, nucleic acids, phosphorus compounds, growth regulators, antibiotics, synthetic and natural food dyes, mycotoxins and pesticides or insecticides in food and water.^[2-4,6,9] For the detection (presence or absence) of the compounds, a developing solution is sprayed on the plate and the spots are visualized. When the substances are not capable of producing color, different techniques can be used such as chemical reactions, adding fluorescent indicators to the adsorbent layer during the process of preparation of the plates or spraying the plates with fluorescent solutions and observing under ultraviolet lamp. For the identification of the substances, the retention factor (Rf) is used. Rf is calculated from the following formula: $Rf = \frac{hx}{h_0}$, where hx is the distance between the starting, or spotting, line (place where spots of samples are applied on the plate in a straight line) and the center of the spot of the substance; and h_0 is the development length that means the distance between the spotting line and the solvent front line (mark of the eluent front, when the eluent approaches the upper edge of the chromatographic plate). On the other hand, for semi-quantitative determination, the intensity of the spot

is used. A larger amount of substance in the mixture is represented by a spot with more intense color. Additionally, the size of the spot can also give semi-quantitative information (a bigger spot indicates a higher content of a particular compound in the mixture). The intensity of the spots is evaluated by comparing with the intensities of spots with known amounts visually or using a densitometer.^[11] In addition, TLC can be one- or two-dimensional similar to other chromatographic techniques such as HPLC, GC and liquid chromatography. In relation to two-dimensional separations (technique performed in two steps), various different coupling strategies have been developed for the first and second steps. The procedure of two-dimensional TLC (2DTLC) begins with the development of a sample-loaded plate with the first solvent. After removing it, the plate is rotated 90° and developed with a second solvent. Developing the plate with two different solvents increases the spot capacity (analogous to peak capacity in HPLC) when compared to single TLC or one-dimensional TLC (1D TLC).^[21] 2D TLC is a powerful tool to separate every complex samples such as lipid mixtures. In general, a solvent mixture with high elution power is used first (dimension number one), followed by an eluent with lower elution power (dimension number two). This confers the advantage that the analyte is concentrated in each step, and the gradient development often helps to overcome problems related to limited resolution. Nevertheless, this method has also serious disadvantages when compared to one-dimensional TLC (1D TLC). Only a single sample can be investigated by using one plate and, thus, 2D TLC is much more time-consuming than 1D TLC. Moreover, as only a single sample can be applied, the simultaneous application of standards is impossible. This makes spot assignments as well as semi-quantitative data analysis highly difficult. Despite these disadvantages, the quality of separation and resolution are highly improved by 2D TLC method which can be used as any other chromatographic technique.^[22]

However, when the claim is rapid separation and visual identification, the TLC is the simplest and most economical between the chromatographic techniques. Therefore, this separation technique was suggested as screening method,^[7] being used in several laboratories for general analysis of foods,^[18] and, due to TLC effectiveness and accuracy, especially in the determination of drug residues and substances from deterioration process like mycotoxins and biogenic amines (histamine, cadaverine, putrescine, spermidine, spermine and others) which are produced from bacterial decarboxylation of the amino acids that naturally compose the foods.^[1,5,8,9] In this context, the laboratory practice in products of animal origin, led to the performing of TLC analyses in response to industrial demand for quality control of fresh and frozen fishes or canned fish, mainly tuna and sardines. These fishes have a significant amount of histidine and, consequently, more pre disposition to histamine formation. Additionally, the

biogenic amines are fairly evaluated in fermented and matured products such as several kinds of cheese and salami, taking into account experimental studies.^[23] In this case, TLC may be used to suggest contamination during the steps of industrial processing or indirectly analyze the hygienic quality of the product or raw material, which is an important characteristic inequality control of products of animal origin.

TLC Methods in Lipids and lipophilicity of compounds

In the field of lipids, TLC is classically used for routine separation and identification of individual lipids.^[26] Regarding the subsequent analysis, the first step is normally the extraction of lipids. This seems rather trivial but it is actually a very important point because many lipids cannot be extracted easily since they are incorporated in the original biological material with other compounds.^[27] For lipid extraction from animal, plant and bacterial tissues the use of chloroform and methanol (2:1, v/v) is recommended because the tissue water is the ternary component and its amount is very important in order to avoid loss of lipids.^[28] The most popular stationary phases for lipid separation are silica gel and alumina, whereby silica gel is unequivocally the absolutely dominant phase. Additionally, silica can be modified by impregnation with other substances to provide optimum results regarding the separation of a certain lipid class.

The TLC method can also be used for the determination of lipophilicity from a single TLC run; however, it is necessary to add an optimal concentration of a well-chosen modifier to the mobile phase.^[3] The mobile phase composition determines the retention time of solutes in both normal-phase and reversed-phase chromatography. The alteration of the composition and the nature of mobile phases enable tuning the retention of the separated analytes and optimization of the chromatographic process.

TLC Methods in Vitamins

Although not all vitamins are amines, they are organic compounds required by humans in small amounts for normal growth, metabolism and health. Human vitamin deficiencies lead to vitaminosis, gastrointestinal problems, cardiovascular and nervous disorders, and growth inhibition.^[185] Therefore, the concentration of vitamins in biological objects, food products and pharmaceutical formulations should be controlled. Water and fat soluble vitamins differ in properties and structure. Vitamins can occur in trace or macroscopic amounts. Some occur in several bioactive forms; for example, vitamin B6 can occur as pyridoxine, pyridoxal, and pyridoxamine. Normal and reversed-phase TLC has been found useful for separation and identification of vitamins.^[186–190] The mobile phases frequently used include mixed organic or aqueous–organic solvents.^[191,192] Most studies have been performed using methanol, ethanol, butanol, acetone, chloroform,

ammonia, pyridine, benzene, or toluene as one of the components of the mobile phase with adsorbents such as silica gel, cellulose, and surface-modified silica gel.^[193-198] For reversed phase TLC of hydrophilic and lipophilic vitamins, RP18 and RP18WF254 HPTLC plates have been used with mixed aqueous-organic mobile phases.^[190,199] In addition to the use of conventional adsorbents, fat soluble vitamins have been separated on TLC plate coated with certain unconventional adsorbents such as corn starch, talc, rice starch etc.^[104] Various dyes have been used to detect D, A and E vitamins for on plate identification after their separation by partition and adsorption TLC.^[200] TLC has also been used to investigate potential interactions between vitamins A and D with frequently used therapeutics.^[201] High sensitivity

of the method allows the separation of pictogram amounts of vitamin D and its analogues on silica gel. A new method has been developed by Lobastova et al.^[202] for the fast screening of synthetic products related to vitamins.

Tocopherol (Vitamin E), a lipid soluble antioxidant is related to many physiological processes and protects polyunsaturated fatty acids in cell membranes from peroxidation.^[203,204] Therefore, the separation of tocopherol isomers has been important. Sliwiok et al. developed a chromatographic method for the separation of tocopherol enantiomers/isomers and provided an interesting relationship between their R_f values and topological indexes.^[205]

Thin layer chromatographic studies of Vitamines, amino acids and their derivatives performed during 1991-2010.

Stationary Phase	Mobile Phase	Remarks	Ref
Silica gel	2-Propanol – 25% ammonia (7:3)	Identification of L-tryptophan by TLC	[116]
Silica	2-Propanol – 25% ammonia (7:3)	TLC of tryptophan	[91]
Silica	Chloroform – methanol – formic acid (35:15:1)	TLC of amino acids. Detection by spraying with 0.2% ninhydrin in ethanol.	[94]
Silica gel	Ethanol – ammonia – water (7:1:2)	Identification of amino acids by TLC. Detection by spraying with ninhydrin.	[106]
Silica gel and RP18	Toluene – pyridine – acetic acid (40:10:1) and aqueous solution of β -cyclodextrin – acetonitrile or methanol	Purification of DNS-amino acids	[118]
Alumina	1% Cetyltrimethyl ammonium bromide in water – butanol (19:1)	TLC of 24 amino acids on alumina and lithium impregnated alumina layers	[115]
Alumina	Oil-in-water microemulsion	Qualitative separation of aliphatic and aromatic amino acids on plain alumina and Li ⁺ , Na ⁺ , NH ₄ ⁺ impregnated alumina with oil-in-water micro emulsion.	[124]
Silica gel	n-Propanol – water (7:3)	TLC of 22 amino acids	[128]
Stationary Phase	Mobile Phase	Remarks	Ref
RP8F254S and RP18F254S TLC plates	Methanol – water	Ergocalciferol, cholecalciferol, ($\pm$)- α tocopherol, tocopherol acetate, retinol, retinol acetate, retinol palmitate, menadione	[234]
Silica gel and kieselguhr	Acetone n-hexane in different volume ratio	Nicotinic acid and its derivatives were separated using adsorption TLC	[231]
Silufol plates high-performance Sorbfil plates	Benzene (first mobile phase); 0.02 M aqueous micellar solution of sodium dodecyl sulfate (second mobile phase)	Fat soluble vitamins A and E and water soluble vitamins B (B1, B2, B6 and B12) can be separated	[228]
Silica gel, mixture of silica gel 60 and kieselguhr	Toluene and methanol	TLC of lipophilic vitamins on silica gel separated	[225]
Silica gel Silica 60F254 TLC plates	n-Hexane – ether (9:1) and benzene – chloroform (1: 1)	Determination of vitamins A, D2 and E from mixtures on fluorescent TLC plates	[224]
Silica gel G	Water	Determination of vitamin B1 (thiamine) in the Kombucha drink.	[217]
Silica plus GDX	Hexadecyl trimethyl ammonium bromide	Resolution of water soluble vitamins B2, B6, and B12 on silica mixed with a polymer packing material	[214]

TLC Methods in Dyes

The synthetic food dyes can also be successfully determined by the TLC technique. Although there is no ideal method for the detection of these compounds, TLC is the best solution to qualitative determination of dyes in a mixture because it allows obtaining satisfactory results in a short time. Moreover, the TLC technique guarantees the correct interpretation of results of analysis because it involves few elements: shape and color of spots, displacement on the plate and light resistance (natural dyes exposed to the light fade in the course of time). For TLC the sample preparation must be carefully purified in order to assure satisfactory separation. Sugars, fats and other substances should be removed by using solid phase extraction (SPE) because partial removal of these compounds causes the blurring of spots.

CONCLUSION

The TLC has a strong potential as a surrogate chromatographic model for qualitative and quantitative analysis. To convert these opportunities into practice, several modifications have been carried out on the conventional TLC system. Therefore, further studies on TLC and HPTLC should be performed in order to contribute with the scientific community and for enhancing the thin-layer chromatography method, facilitating the laboratory practices due to performance and economic advantages compared to other chromatographic techniques such as paper chromatography, HPLC and gas chromatography.

Future Prospects

TLC coupling to other analytical techniques and its application to the analysis of real samples as well as purification will continue. The search for new layer materials, automation in quantitative analysis, and validation of TLC parameters will be the priority of chromatographers.

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