For analysis, a CN column was used with acetone–0.01 M KH_2PO_4 (1 : 5, v/v) as a mobile phase.

See also: **II/Chromatography: Liquid:** Mechanisms: Reversed Phases. **III/Food Additives:** Thin-Layer (Planar) Chromatography.

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Thin-Layer (Planar) Chromatography

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Introduction

Thin-layer chromatography (TLC) is a relatively old technique among the other chromatographic separation methods. In food additive analysis, this simple technique is the tool of choice, mainly because the high throughput of samples that it can manage in parallel and the wide range of compounds that can be analysed simultaneously.

Food Additives

Anything added to food is not necessarily a food additive. Generally, a food additive is a substance or a mixture of substances different to the bulk of the food and present as a result of any aspect of production, processing or packing. This definition does not include hazardous contaminants.

The Codex Alimentarius Commission for Food Additives defines these as follows:

> **Food additive** means any substance not normally consumed as a food by itself and normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such foods, results or may reasonably expect to result (directly or indirectly), in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include 'contaminant' or substances added to the food for maintaining or improving nutritional qualities.
>
> Others definitions include:
>
> Substance with non-nutritive properties, known chemical composition, intentionally added to food; generally in small amounts, with the aim of improving presentation (appearance, flavour, texture) and conservation properties of foods.

In other countries, such as Spain, additives are all substances that can be added intentionally to food and drink, without the purpose of changing the nutritive value, to modify processing and conservation characteristics, as well as to improve their adaptation to the use for which it is produced.

Classification of Food Additives

Many methods have been used to classify food additives. The majority imply functional grouping.

Chemical-type grouping is convenient because it puts together moieties of similar structures and chemical properties in comparative categories. Toxicological and metabolic studies can also be correlated with chemical grouping. However, compounds belonging to the same chemical family have different functions in the food industry. In spite of the fact that a compound can have two or more different functional groups, this classification is more practical in the food industry. **Table 1** shows a typical classification of food additives.

Table 1 shows the diversity of compounds included in the different classes generated. Grouping by functional group type can include chemical substances both naturally and structurally quite different. This is an additional problem for the analysis of substances considered, classified or included in lists of food additives.

In modern quality control, analysis is required at every step and not just in the final product. This is to prevent possible defects directly at the critical points but it produces a significant increase in the number of samples and the number of analyses to be carried out.

On the other hand, it is important to consider that additives can be applied exclusively to those foods where regulation points out specifically that they must be used and normally they must be declared on labels attached to the food.

In spite of tolerance limits for some additives, the amount added should not exceed the amount adequate to attain the objective, using the appropriate manufacturing procedure. This justifies the necessity to detect and quantify food additives. Today there are many analytical procedures applied to these substances. Obviously the method used will depend on the analytes, and their characteristic and/or physicochemical properties.

Table 1 Permitted classes of food additives in Australia

Class of additive	*Property of food influenced*
Preservatives	Shelf-life
Colourings	Appearance
Flavouring and flavour enhancers	Flavour
Antioxidants	Shelf-life
Artificial sweetening substances	Flavour, energy value
Vitamins and minerals	Nutritive value
Modifying agents	
Vegetable gums	Texture, appearance
Mineral salts	Texture, appearance
Food acids	Shelf-life, flavour, texture
Emulsifiers	Texture, appearance
Humectants	Texture, shelf-life
Thickeners	Texture

It is necessary to extract the additive compound from the food matrix and to apply additional purification procedures where necessary. Chromatographic methods, which involve a separation process, allow the isolation of the compound(s) to be analysed. High performance liquid chromatography (HPLC), gas chromatography (GC) and TLC have all been used extensively for the final analysis.

For large numbers of samples, the comparative advantage of TLC is that it is a completely instrumental technique that can deal with many samples simultaneously, and with samples of a diverse nature.

In the past few years, many reviews have been published with the aim of featuring the relevant characteristics of instrumental planar chromatography, or high performance thin-layer chromatography (HPTLC).

In the first place, it is necessary to describe the advances obtained in the preparation of sorbents for stationary phases.

Silica still represents the most frequently used material for the stationary phase. Approximately 90% of separations by TLC are still carried out using silica. Modern sorbents are characterized by smaller and more uniform particle size, implying a reduction of equivalent plate height. This results in a higher number of theoretical plates for a given run length compared with traditional phases and, in turn, this allows separations in shorter distances with corresponding time savings and reduction of diffusion problems that appear when the mobile phase is retarded excessively.

Stationary phases of polarity from intermediate to reversed phase, have been developed. Most of them are obtained by chemical modification of silica gel (**Table 2**).

Recently, sorbents with spherical particles such as Lichrospher Si 60 F254s have appeared. They offer shorter analysis times, improved separation

Table 2 HPTLC stationary phases used in food additives analysis

Stationary phase	*Food additives*
3-Aminopropyl	Sugars, carboxylic acids, preservatives
Reversed-phase, C18	Preservatives, antioxidants
Aluminium oxide	Lipophilic food dyes
Silica normal	Antioxidants, sweeteners, surfactants, dyes
Cellulose	Artificial sweeteners, carboxylic acids
Cellulose MN 300	Food dyes

efficiency, more compact spots, higher spot capacity and lower detection limits. All these new plates are applicable to the analysis of many analytes including those used as food additives.

Advances in instrumentation in the whole chromatographic process, ranging from application devices through automatic developing chambers, and automated multiple development (AMD) chambers with computer control, all deserve special consideration. Computer-controlled AMD using polarity gradients increases efficiency in separation to limits comparable to HPLC, and retains the high throughput of samples to be analysed in the same chromatographic run.

Major developments in densitometers have meant improvements in the quantitative analytical ability of planar chromatography. These, coupled to software, allow quantitative analysis of substances at very low concentrations thanks to the high sensitivity of detectors that allow measurements over the whole UV-visible spectrum as well as fluorescence.

Another important aspect of HPTLC in food additive analysis, is that there are some compounds with difficult detection characteristics. The variety of reagents available, overcomes this difficulty and they can be used as pre- or postchromatographic derivatizing agents.

Finally, video scanning not only allows information to be saved digitally but also gives quantitative results from image integration.

Unlike HPLC, TLC needs very little sample purification and can be used with raw or dirty materials, thereby saving time and additional expense.

Applications of HPTLC in Food Additive Analysis

TLC has been used for many years in the analysis of food additives, such as food dyes, preservatives (**Table 3**), antioxidants and sweeteners.

Food colourants are in many cases fundamental food additives because consumers judge product quality by its colour. On the other hand, before a dye (natural or synthetic) is permitted for use on food it has to be shown that it is nontoxic and noncarcinogenic. The current list in western European countries comprises about 30 natural or artificial substances permitted as food dyes, and is very small compared to the vast number of known dyes. This makes it necessary to have easy and fast methods to detect forbidden or unapproved food dyes (**Table 4**).

Because the importance of colourings in foods, a variety of separation procedures are still being examined with the aim of improving performance.

Table 3 Analysis of preservatives by HPTLC

Preservatives	*Layer*	*Eluent*	*Detection*	*Reference*
p-Hydroxybenzoates n-propyl, ethyl, methyl	Silanized silica C18	Methanol–water (7 + 3, 6 + 4, 5 + 5, 4 + 6 v/v)	UV $\lambda = 270$ nm	Volkmann D (1980) *HRC and CC*, 3: 189
8 different preservatives	Mixed layers of silica and cellulose, F254	Petroleum ether–CCl_4–$CHCl_3$–formic acid–glacial acetic acid (50 + 40 + 20 + 8 + 2 v/v)	UV $\lambda = 254$ nm	Gosselé JAW (1971) *Journal of Chromatography* 63: 433
Propyl, ethyl, methyl, hydroxybenzoates, 4- hydroxybenzoic acid	RP18 W/F254	Acetone–water (40 + 60 v/v)	UV $\lambda = 254$ nm	Machery-Nagel (1990) TLC department
Parabens (hydroxybenzoic acid esters)	Silicagel 60, F254	1) pentane–dichloromethane–acetic acid (25 + 25 + 3 v/v) 2) petroleum ether–diethyl ketone–acetic acid (88 + 5 + 12, v/v)	UV $\lambda = 255$ and 310 nm	Zimmermann A *8th Symposium of German Association of Scientific and Applied Cosmetics,* Hamburg, November 1989
Benzoic acid, sorbic acid and parabens	Polyamide/cellulose	Toluene–petroleum ether–$CHCl_3$–acetic acid (30 + 15 + 10 + 1 v/v)	UV $\lambda = 230$ benzoic acid $\lambda = 260$ others	Duden R, Frikers R, Calverley KH, Park, Rios VM, Lebensm Z (1973) *Unters, und Forsch* 151: 23

Table 4 HPTLC systems for food dye analysis

Food dye	*Layer*	*Eluent*	*Detection*	*Reference*
Seven dyes: erythrosine, brilliant black NN, fast red E, naphtol red, yellow orange S, ponceau 4R, tartrazine	Cellulose MN300	Sodium citrate 2.5%–25% ammonia–methanol (20 + 5 + 3, v/v/v)	Coloured substances	Machery-Nagel (1990) TLC Department
Tartrazine, Amaranth Indigo Carmine, New coccine, Sunset yellow FCF, Allura Red Ac, Fast green FCF, Brilliant blue FCF, R-106, R-103, R-3, R-105, and R-104	Silica-RP18	(A) methanol–acetonitrile–5% sodium sulfate (3 + 3 + 10, v/v/v) (B) methanol–MEK–5% sodium sulfate (1 + 1 + 1, v/v/v)	Scanning at different wavelengths	Oka H *et al.* (1987) *Journal of Chromatography* 411: 437–444
Sulfonated dyes ponceau, tartrazine, azorubin etc.	RP18; ion pair optimization	(A) Methanol–water (8 + 2, v/v) (B) Methanol–water + 20 mM solution of tetrabutylammonium bromide	Visual comparison	Korner A (1993) *Journal of Planar Chromatography* 8: 138–143
Unlawful food dyes detection	Silica-RP18	(A) methanol–acetonitrile–5% sodium sulfate (3 + 3 + 10, v/v/v) (B) Methanol–MEK–5% sodium sulfate (1 + 1 + 1, v/v/v)	TLC–FAB–MS	Oka H *et al.* (1994) *Journal of Chromatography A* 674: 301–307
Quinoline Yellow, Sunset Yellow, Cochineal Red A, Indigo Carmine, Tartrazine, Amaranth, Erythrozine	Silica 60, F254 OPLC	(A) NH_3–methanol–ethyl acetate (1 + 3 + 6, v/v/v) (B) NH_3–MEK–n-butanol (2 + 3 + 5, v/v/v)	Different wavelengths	Rózylo JK and Siembida KR (1997) *Proceedings of the 9th International Symposium on Instrumental Planar Chromatography*

The use of surfactants, (hexadecyltrimethylammonium bromide) incorporated in the mobile phase for the separation of acids and alkaline food colourings, new polymer coatings for plates and new adsorbents such as Scolecite (corresponding to a natural zeolite) are recent approaches. Some colourants such as indigo carmine, cochineal red, acid amaranth and tartrazine G, have been separated on thin magnesium oxide layers with mixtures of 15% sodium citrate and methanol. Reversed-phase plates, obtained by impregnation of silica plates with 10% liquid paraffin in petrol ether are used for separation of different food dyes with advantage.

For food preservatives like benzoic and sorbic acid, the use of methods such as solid phase extraction (SPE) allow a better separation of these food additives from natural ingredients present in beverages.

Overpressured thin layer chromatography (OPTLC) has been shown to be a good tool for the separation of a variety of food additives, like antioxidants, natural food colourings, preservatives and water-soluble vitamins with silica plates. In this field, AMD has also been successful in the separation, identification and quantification of a diverse range of antioxidants.

Other food additives that requires adequate control are artificial sweeteners and antioxidants, because of the carcinogenic properties attributed to some of them. Analysis of these is frequently carried out by HPLC, but, HPTLC shows the comparative advantages formerly mentioned. **Table 5** summarizes some HPTLC systems for analysis of these food additives.

See also: **II/Chromatography: Thin-Layer (Planar):** Layers; Modes of Development: Forced Flow; Over Pressured Layer Chromatography and Centrifugal; Spray Reagents. **Dyes:** High-speed Countercurrent Chromatography; Liquid Chromatography; Thin-Layer (Planar)

Table 5 HPTLC systems for antioxidants and sweeteners

Additive	*Layer*	*Eluent**	*Detection*	*Reference*
BHA, BHT, NDGA, Gallic acid esters	Silicagel 60, F254 OPLC	$CHCl_3$–HAc $CHCl_3$–Methanol–HAc Benzene-Methanol–acetone–HAc Methanol–acetone–water	Spraying with 0.5% solution of 2,6 dichloroquinone–4 chlorimide and heating to 105°C	Siembida R (1997) *Proceedings of the 9th International Symposium on Instrumental Planar Chromatography*
Gallic acid esters, BHA, BHT, DBH, TBH	Silicagel-G25 HR	Petroleum ether–benzene–HAc (2 + 2 + 1, v/v/v)	Spraying with 0.5% solution of 2,6 dichloroquinone–4 chlorimide and heating to 105°C	Machery-Nagel (1990) TLC Department
BHA and dodecylgallate	Silicagel	Xylene–$CHCl_3$–propanol–formic acid–HAc (45 + 45 + 10 + 1 + 1, v/v/v/v/v)	10% Phosphomolybdate or vainillin in sulfuric acid	Sherma J and Fried B (1991) *Handbook of Thin Layer Chromatography.* Marcel Dekker, Inc.: 702
Saccharin, cyclamate	Laboratory made mixed layers Cellulose MN300–polyamide; (3 + 2)	Xylene–HAc–n–propanol–formic acid (45 + 7 + 6 + 2 v/v)	Spray of developed plate with ethanolic dichlorofluorescein solution	Wooldich *et al.* (1969) *Z Lebenm. Unters. Forsch.* 139: 142
Aspartam, acesulfam, saccharin	Silicagel G-25, UV_{254}	Xylene–HAc–n–propanol–formic acid (45 + 7 + 6 + 2 v/v)	Scanner dual wavelength 215/370 nm	Machery-Nagel (1990) TLC Department

* HAc = acetic acid

Chromatography. **Food Additives:** Liquid Chromatography. **Impregnation Techniques: Thin Layer (Planar) Chromatography. Pigments:** Liquid Chromatography; Thin-Layer (Planar) Chromatography.

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