JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2011, 17(1), 46-53

Journal of Agroalimentary Processes and Technologies

Separation and identification of some synthetic food colorants from foods through thin-layer chromatography – "UV- VIS" Spectrometry

Olivia Florena Măruțoiu[1], Ioan Gogoaşă[2*], Constantin Măruțoiu[3], Maria Tofană[4], Diana Moigrădean[2], Iosif Gergen[2]

[1]*„Babes-Bolyai" University, Faculty of Chemistry and Chemical Engineering, Cluj-Napoca, Arany Janos stret, no. 11 , Romania*
[2]*Banat's University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Proccessing Technology, Timişoara, Aradului street, no. 119*
[3]*„Babes-Bolyai" University, Faculty of Orthodox Divinity, Cluj-Napoca, Avram Iancu street, no.18, Romania*
[3] *University of Agricultural Sciences and Veterinary Medicine, Agricultural Faculty, Cluj-Napoca, Mănăştu, street, no.3-5, Romania*

Received: 14 December 2010; Accepted: 10 Ianuary 2011

Abstract

A mixture of ten synthesis food colorants was separated on chromatographic plates with diatomitic n-octylsilane (C-8) and n-octadecylsilane (C-18) chemically altered diatomitic earth plates using as a mobile phase an absolute ethanol mixture – K_2SO_4 1% in water (40:60, v/v), and absolute ethanol – K_2SO_4 0.5% in water (40:60, v/v), respectively. Samples of non-alcoholic beverages with different colorants were evaporated to dry and the residue was dissolved in 0.5 mL ethylic alcohol and chromatographed together with samples on chemically altered diatomitic earth plates. Identifying synthesis colorants in foods was done on the ground of samples and through UV-VIS adsorption spectrometry after cutting out the spots and extracting the colorants in methanol-water.

Keywords: food colorants, through thin-layer chromatography, uv-vis spectrometry

1. Introduction

The colour of foods is given by the organic compounds present in their composition and that contain in their molecule some groups carrying colour (chromophorous groups). The main chromophorous groups are: NO- (nitroso), NO_2- (nitro), -N=N- (azo), CO- (carbonyl), =C=O (ketone), =C=N- (azometine), =C=S (tio-ketone), =C=C= (conjugate), -C=C-, =C-O, -N=O.

The presence, in the molecule of the organic compound of two or more conjugated double bonds (conjugate chromophores) gives birth to a vivid colour. Colour is a feature of substances and it occurs as a result of the interaction between light and substances.

This feature can usually be perceived by humans directly or with apparatuses. Colour or, more precisely, colour sight also has a subjective character since it depends largely on the eye of the beholder. Human eye detects colour only on a very narrow range of electromagnetic radiation, i.e. in the visible range between 400 and 800 nm. The chromatic colour of substances corresponding to coloured radiation in the visible range is determined by the absorption, reflexion, and/or selective transmission of radiation in the visible range. The colours white, black, and grey are not due to absorption and/or to the selective reflexion of electromagnetic radiation in the visible range and are, therefore, called achromatic colours.

Corresponding author: *e-mail:* ionelgogoasa@yahoo.com

These colours are due to non-selective absorption, more exactly to absorption or to constant total/partial reflexion in the entire visible range [1].

The human eye perceives white light as a result of its action on the mixture of all coloured radiation in the visible range or of the mixture of two such colours. The pairs of coloured radiation in the visible range whose mixture is perceived by the eyes as light sensation are called complementary radiation.

When a compound or an object absorbs a radiation of the spectrum, it has the colour of complementary radiation. Thus, are complementary the purple and yellow radiations. Radiations in the visible range are characterised by wave lengths, i.e. by their colours. Thus, each colour corresponds to certain wave lengths (purple 400-440 nm, blue 440-500, green 500-570 nm, yellow 570-610 nm, orange 610-670 nm, and red 670-800 nm). Thus, by recording the absorption spectrum in the visible range one can identify the corresponding colorant [1].

Synthetic colorants cover a numerous number of organic and inorganic salts, as well as thousands of organic-metallic compounds. In most existing colorants, there is a number for each colour index (CI, see Table 1) that help identifying its structure and features, as well the identity of a colour when manufacturers use several names for the same colorant [2].

Synthetic pigments and colorants have various uses in society. They are used in the manufacture of printing inks, in colouring textiles, in the manufacture of plastics and of cosmetics, in colouring histological and cystological preparations in painting and in the food industry.

The colorants used as additives in the food industry to colour foods play a commercial and aesthetic role making foods more attractive and marketable, playing a major psychological role in food choice – with colour an indicator of quality. Food colorants should satisfy certain requirements concerning the transparency degree, light resistance, toxicity, etc. They are added in small amounts and usually lack any nutritious value. From the point of view of the way they are obtained, food colorants are classified as natural and synthetic. Due to the low price, synthetic colorants are the most used in the food industry though consumers prefer natural colorants [3].

Along the years, the assortment of colorants used was diversified but their use started to be restricted because of their toxicity. This is obvious in the legislation of many countries in which the number of food colorants authorized for use was considerable reduced (in the U.S.A., at the beginning of the years 1900, they used 80 food colorants, while nowadays they use less than 26) or even forbidden (Norway) [4, 5].

In all the fields of use, it is purity that counts and, in certain cases, it is the presence or absence of certain impurities or intermediaries. In all these cases, thin-layer chromatography proved the ideal solution since it can separate and identify related colorants and intermediaries [6]. This is possible due to the wide range of stationary phase, almost unlimited possible variations of the mixtures of the mobile phase. The only limitations of solvent mixtures are represented by the adverse effects on the adsorbent layer, by the reaction with chemically altered phases, and by the high viscosity and surface tension of the solvents.

The use of plane chromatography in colour analysis has, compared to other chromatographical techniques, a series of advantages among which the fact that the substances separated are easy to see due to their colour. It happens that slight differences in hue are clearly more visible on layer than in solution. Thin-layer detection limits are within the range of picogrammes-nanogrammes. Due to the fact that, on a single chromatographical plate, one can apply several samples to be analysed together with other control samples and the analysis can be done in a single developing stage, the cost per sample is significantly diminished compared to liquid chromatography.

In most chromatographical techniques, analysis of these compounds is done by extracting the colorant from any pattern and further treatment of the sample is time-consuming, it is a necessary one because impure colorants contain many substances that are easily retained by the chromatographical adsorbents in the columns. Liquid chromatography pre-columns and columns become useless because of their charge with substances they should be either discharged, or laboriously cleaned with a solvent before re-use. In thin-layer chromatography, we need to minimally purify the extract since most polar impurities stay close to the start and non-polar impurities migrate with the solvent front (in the separation in normal phase).

Since the developed chromatographical plate is not re-used, the material not separated from the start or from the solvent front is not important. Nevertheless, the chromatogrammes thus obtained contain rich data concerning colorant separation. Both colorants and the rest of the separated compounds can be scanned through spectrodensitometry, but one can also record individual spectra in the visible or ultraviolet light of the areas separated chromatographically using a series of other spectroscopic techniques such as Fourier transform infrared spectroscopy (FTIR), mass spectrometry (MS), nuclear magnetic resonance (NMR), and Raman spectrometry.

A number of 20 food colorants present in foods, medicines, and cosmetics were separated on silica gel using as a mobile phase the mixture of ethanol-methanol-water-ammonia hydroxide and acetate (150:40:35:5, v/v). The sample was treated with concentrated phosphoric acid diluted with methanol and followed by neutralisation with ammonia and centrifugation to separate the liquid phase. The extract thus obtained was applied on silica gel chromatographic plates together with standard samples [7]. A mixture of V patent blue, quinoleine yellow, FCF brilliant blue, tartrasin, azorubine, 4R ponceau, curcumin, indigo, carmine, cochineal, violet methyl, mixtures of carotenes, caramel (erythrosine B) and orange yellow S were separated on G silica gel with isopropanol/ammonium hydroxide 12.5% (10:2, v/v) as a mobile phase. This chromatographical system was applied to 23 food additives used in carbonated and non-carbonated beverages [8]. Gertrud E. Morlok and Claudia Oellig [9] developed a quick method of separation, identification, and quantitative determination of 25 soluble colorants in water used as food additives. Separations were made on $60F_{254}$ silica gel plates in rooms with horizontal developing using as a mobile phase a mixture of ethyl acetate- methanol-water-acetic acid (65:23:11:1,v/v) over a migration distance of 50 mm. Identifying colorants was done with UV-VIS spectra and of mass spectrometry.

Extraction in solid phase and thin-layer chromatography was used in the qualitative and quantitative analysis of beverage colorants. The separations were made in normal phase on silica gel and silica gel altered diol, cyanopropyl, aminopropyl, and on inverse phase on silica gel altered octadecyl, diol, aminopropyl, and cyanopropyl.

As a mobile phase, we used different mixtures of organic solvents (tetrahydrofuran, dioxane, methanol, acetonitrile, and acetone) with amines, ammonia hydroxide buffer solutions [10]. A number of 9 colorants soluble in water were separated from foods through thin-layer chromatography with inverse phase. Separations were made in horizontal development room on RP-18 plates with silica gel using as a mobile phase mixtures of ethanol or acetone with aqueous solutions of ammonia sulphate (0.1; 0.5 and 1 $mol.L^{-1}$) [11]. Harada *et al*. [12] separated 11 food colorants on chemically altered silica gel C-18 with methanol-methyl-ethyl-cetone-10% Na_2SO_4 in water (1:1:3.5, v/v) or methanol-acetonitrile-aqueous solution 10% Na_2SO_4 (3:3:10, v/v) as mobile phases and identification through liquid secondary ion mass spectrometry (LSIMS). Food colorants were determined quantitatively through digital processing of images after thin-layer chromatography on silica gel plates $–NH_2/UV_{254}$ using as a mobile phase a mixture of isopropanol-ethylic ether-ammonia (2:2:1, v/v) [13].

Food colorants were separated on other types of adsorbents on thin-layer chromatography as well. Thus, we separated 4 food colorants on layers of zheolyth using as a mobile phase acetone [14], MgO layer using as eluent a mixture of methanol-sodium citrate 15% (8:2, v/v) [15], DEAE-cellulose layer, PAB-cellulose layer, silica gel layer impregnated with cationic and anionic detergents [16], as well as other stationary phases [17]. Starting from these data, we aimed at developing a separation and identification method of some food colorants from different non-alcoholic beverages through thin-layer chromatography and UV-VIS spectrophotometry.

2. Material and Method

We prepared thin layers (0.25 mm) on glass plates (10 x 10 cm) using as adsorbent C-8 and C-18 chemically altered diatomitic earth plates, respectively. We prepared thin layers through mixing chemically altered diatomitic earth plates altered with fluorescence indicators (barium aluminate and magnesium aluminate activated with europium + yttrium aluminate activated with cerium) that have a whit fluorescence over the range 254-366 nm after which we added o solution of poly-vinyl-pyrolidine in ethylic alcohol [18].

The paste thus obtained was laid on washed and degreased glass plates after which the chromatographical plates thus prepared were set to dry at room temperature.

On the plates thus prepared, we applied solutions 0.01% in ethanol-water (1:1, v/v) from the food colorants presented in Table 1 in volumes of 5µL/spot with a Brand micro-dripper together with 5 µL/spot samples of non-alcoholic beverages. The plates were developed in an N unsaturated room through the ascending technique using as a mobile phase absolute ethylic alcohol (99.6%) – K_2SO_4 1% in water (40:60, v/v) for the C-8 chemically altered diatomitic earth plates and absolute ethylic alcohol (99.6%) – K_2SO_4 0.5% in water (40:60, v/v) for the C-18 chemically altered diatomitic earth plates. The process of separation was stopped after the colorants migrated over a distance of 7 cm. The colorants separated from beverage samples were identified on the ground of standards and through extraction with methyl alcohol-water (1:1, v/v) from the spot scratched from the chromatographic plate and analysed through UV-VIS spectroscopy. To do so, we used a UV-VIS CT-5 (JASCO) spectrophotometer.

Table 1. Studied food colorants

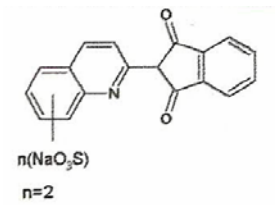
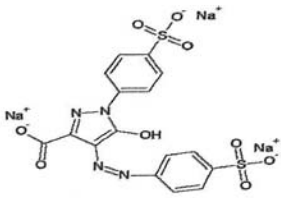
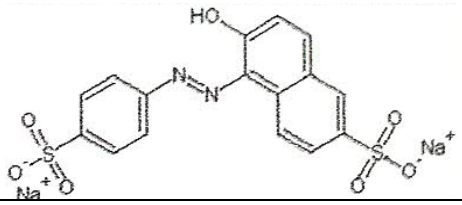
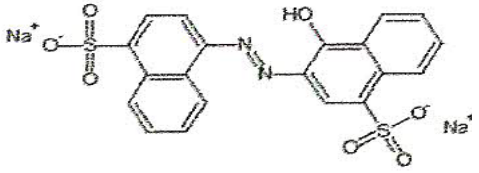
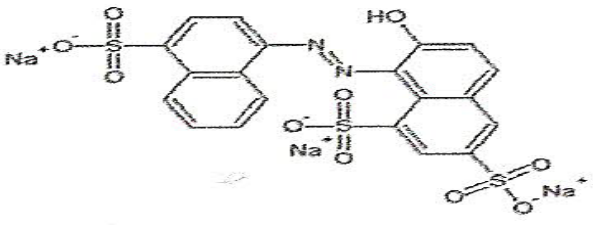
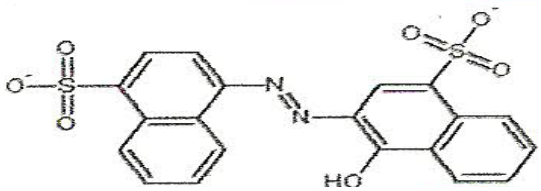
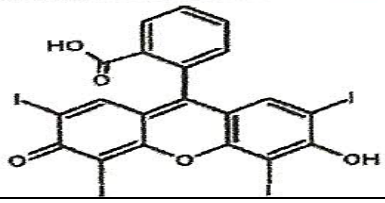
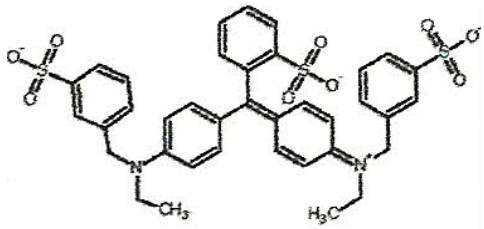
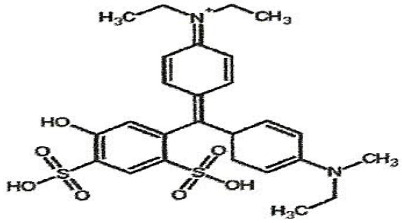
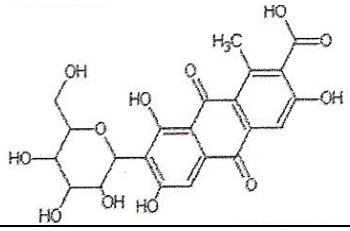
No.	Colorant	Number E	Structural formula
1	Galben de qinolină (quinoline yellow)	E 104	
2	Tartrazină (tartrazine)	E 102	
3	Galben strălucitor FCF (bright yellow FCF)	E 110	
4	Azorubină (azorubine)	E 122	
5	Ponceau 4R (ponceau 4R)	E 124	

Table 1. Studied food colorants (*Continue*)

No.	Colorant	Number E	Structural formula
6	Amarant (amaranth)	E 123	
7	Eritrozină (eritrozine)	E 127	
8	Albastru brilliant (brilliant blue)	E 133	
9	Albastru patent V (patent blue V)	E 131	
10	Carmine, acid carminic, cochineal	E 120	

3. Results and Discussion

As shown in Figure 1, four food colorants are removed from the upper side of the chromatographic plate and the other five are in the lower side and re separated.

Seven of the nine colorants are separated on the studied chromatographic system. In the sample of non-alcoholic beverage we could also identify after the colour and the values of R_F the food colorants quinoline yellow and tartrasin that correspond to the standards (1 and 2).

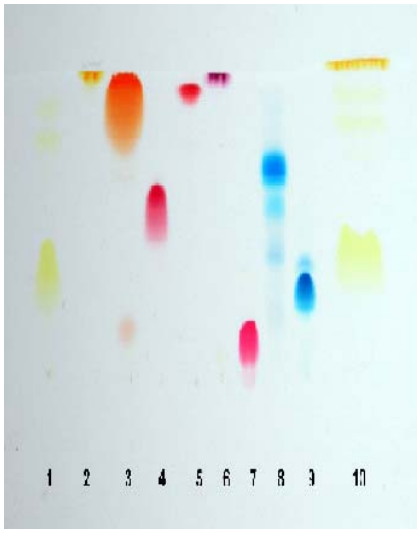


Figure 1. Chromatogramme of the separation of food colorants on C-8 chemically altered diatomitic earth plates using as a mobile phase absolute ethanol – K_2SO_4 1% in water (40:60, v/v),1-quinoline yellow, 2-tartrazine, 3-bright yellow FCF, 4-azorubine, 5-ponceau 4R, 6-eritrozine, 7-amaranth, 8-brilliant blue, 9-V patent blue, 10-extract of non-alcoholic beverage of the separation of food colorants on C-18 chemically altered diatomitic earth plates using as a mobile phase absolute ethanol – K_2SO_4 0.5% in water (40:60, v/v),1-quinoline yellow, 2-tartrazine, 3-bright yellow FCF, 4-azorubine, 5-ponceau 4R, 6-eritrozine, 7-amaranth, 8-brilliant blue, 9-V patent blue, 10-carmine, 11-extract of non-alcoholic beverage

Figure 2. Chromatogramme of the separation of food colorants on C-18 chemically altered diatomitic earth plates using as a mobile phase absolute ethanol – K_2SO_4 0.5% in water (40:60, v/v),1-quinoline yellow, 2-tartrazine, 3-bright yellow FCF, 4-azorubine, 5-ponceau 4R, 6- eritrozine, 7-amaranth, 8-brilliant blue, 9-V patent blue, 10-carmine, 11-extract of non-alcoholic beverage

To confirm this, we scratched the coloured spots from the sample and the colorants were extracted with a mixture of ethanol-water (1:1, v/v) and analysed through UV-VIS spectrophotometry. Results are presented in figures 3 and 4. Absorption spectra of the samples confirm the fact that the two yellow colorants in the sample are quinoline yellow and tartrazine.

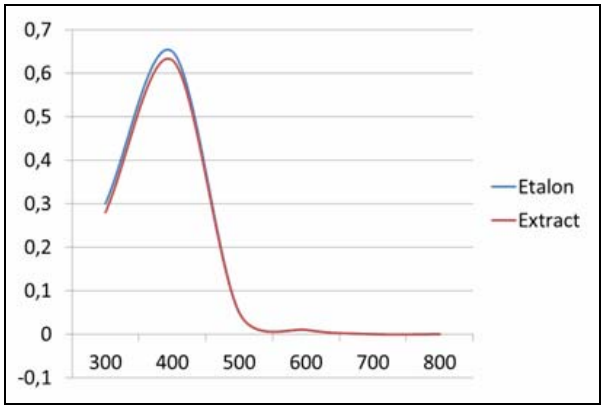


Figure 3. UV-VIS absorption spectra of the food colorant quinoline yellow and of the sample extract after separation on chromatographic plates

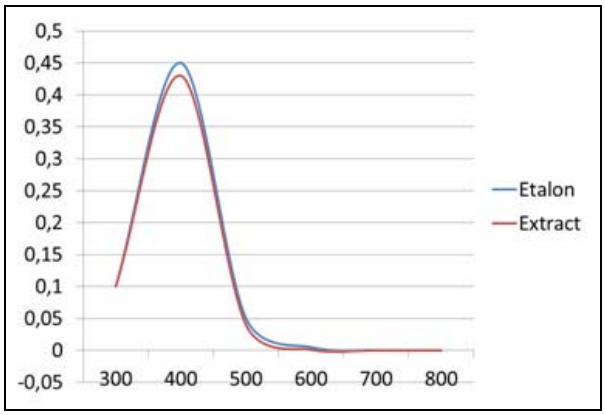


Figure 4. UV-VIS absorption spectra of the food colorant tartrazine and of the sample extract after separation on chromatographic plates

Using C-18 chemically altered diatomitic earth plates and as a mobile phase absolute ethanol – K_2SO_4 0.5% in water (40:60, v/v) separations are better, and eight of the ten colorants are separated. We used several colorants as standards since one of the colorants in the sample to analyse was carmine. In the red-coloured non-alcoholic beverage sample, we identified the colorant azorubine both through chromatography (figure 2) and UV-VIS spectrophotometry (figure 5).

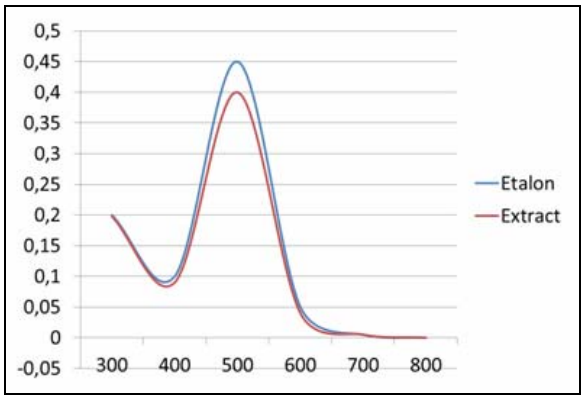


Figure 5. UV-VIS absorption spectra of the food colorant azorubine and of the sample extract after separation on chromatographic plates

In the chromatographic separations in figure 1 and in figure 2, respectively, we can see that in certain colorants there are supplementary spots to the basic spot. Thus, besides the quinoline yellow there are also two yellow spots, in the bright yellow a red spot, in the brilliant blue other five, and in the V patent blue, one more spot. This shows that in food colorants there are many impurities that could be toxic.

4. Conclusions

Using C-8 and C-18 chemically altered diatomitic earth plates as stationary phase and a mixture of absolute ethanol (99.6%)-aqueous solution – K_2SO_4 in different concentrations (40:60, v/v) as a mobile phase food colorants present in different foods can be separated and their purity can be determined.

Besides the colour of the spots and the value of the $R_{F,}$ UV-VIS spectrophotometry can supply valuable information in the identification of food colorants in different foods.

Acknowledgements
The authors wish to thank for the financial support provided from programs co-financed by The Sectoral operational programme human resources development, Contract POSDRU 6/1.5/S/3 – „Doctoral studies: through science towards society".

References

1. Panea, I., *Coloranți organici*, Editura Presa Universitară Clujeană, Cluj-Napoca, 2007
2. *The Colour Index*, 3rd edn Bradford, UK: Society of Dyes and Colourists, 1982
3. Tofană Maria, *Aditivi Alimentari/Interacțiunea cu Alimentul*, Editura Academic Pres, Cluj-Napoca, 2006
4. Shibamoto, T., Bjeldanes, L.F., *Introduction to Food Toxicology*, Second Edition, Elsevier, Amsterdam, 2009, p.247
5. Izbirak, S., Sumer, S., Diril, N., *Mutagenicity testing of some azo dyes used as food additives*, Bulletin of Mikrobiology, **1990**, *24*(1), 48-56
6. Gupta,V.K., Synthetic dyes. In: Sherma J. and Fried B.(eds.), *Handbook of Thin-layer Chromatography,* Marcel Dekker, New York, 1990, pp. 939-969
7. Fadil, F.A., McSharry, W.O., Extraction and TLC separation of food, drug, and cosmetic dyes from tablet-coating formulations*, J. Pharm. Sci.*, **1979**, *68*(1), 97-98, doi: 10.1002/jps.2600680131
8. Baranowska, I., Zydron, M., Szczepanik, K., TLC in the Analysis of Food Additives, *J.Planar Chromatogr-Mod.TLC*, **2004**, *17*(1), 54-57, doi. 10.1556/JPC.17.2004.1.12
9. Morlok, E.G., Oellig, C., Rapid Planar Chromatographic Analysis of 25 Water - Soluble Dyes Used as Food Additives, *JAOAC International*, **2009**, *92*(3), 745-756
10. Tuzimski, T., Wozniak, A., Application of solid-phase extraction and planar chromatography with diode-array detection to the qualitative and quantitative analysis of dyes in beverages, *J.Planar Chromatogr.-Mod.TLC*, **2008**, *21*(2), 89-96, doi: 10.1556/JPC.21.2008.2.3
11. Milojkovic-Opsenica, D.M., Lazarevic, K., Ivackovic, V., Tesic, Z.Lj., Reversed-Phase Thin-Layer Chromatography of Some Foodstuff Dyes, *J.Planar Chromatogr.-Mod.TLC*, **2003**, *16*(4), 276-279, doi: 10.1556/JPC.16.2003.4.5
12. Harada, K.I., Masuda, K., Suzuki, M., Oka, H., Separation and identification of food dyes by thin-layer chromatography/liquid secondary ion mass spectrometry, *Biological Mass Spectrometry*, **1991**, *20*(9), 522-528, doi: 10.1002/bms.1200200904
13. Soponar F., Moț A.C., Sârbu C., Quantitative determination of some food dyes using digital processing of images obtained by thin-layer chromatography, *J. of Chromatography A*, **2008**, *1188*(2), 295-300, doi:10.1016/j.chroma.2008.02.077
14. Shrivastava P.K., Prakash R., Thin layer chromatographzic separation of a few food dyes over scolecite as a new adsorbent, *Chromatographia*, **1986**, *21*(11), 655-656, doi: 10.1007/BF02311923

15. Mastowska, J., A new chromatographic method for the separation of food dye mixtures on thin MgO layers, *Chromatographia*, **1985**, *20*(2), 99-101, doi: 10.1007/BF02280605
16. Lepri, L., Desideri, P.G., Coas, V., Separation and identification of water-solubile food dyes by ion-exchange and soap thin-layer chromatography, *J. Chromatogr.*, **1978**, *161*(21), 279-286, doi:10.1016/S0021-9673(01)85237-7
17. Kucharska M., Grabka, J., A review of chromatographic methods for determination of synthetic food dyes, *Talanta*, **2010**, *80*(3), 1045-1051, doi:10.1016/j.talanta.2009.09.032
18. Marutoiu, C., Coman, M.V., Patent Romania, nr.113278 B, 1998