Article

Separation and determination of some food dyes in soft drinks by RP-HPLC

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Abstract

In this work, an easy and accurate method was accomplished for measuring, determining, and separating some dyes used in food products, which are (E102), (E110) and (E122) using RP-HPLC technique. The C18 column (MN comp.) was used to analyze. The previously mentioned dyes. The calibration curves were conducted at the concentrations range of (1-30) ppm. The LOD and LOQ were calculated, which their values were (0.905-2.26 µg/mL) and (2.743-6.85 µg/mL) respectively, In this study, A mixture of food dyes was separated using isostatic and gradient RP-HPLC modes. The method was applied to samples of soft drinks from local markets in Baghdad- Iraq. They were used to estimate the amount of food dyes they contain, and from the results we can judge that the method was successful and good.

Key words: Separation, RP-HPLC, Food dyes.

Introduction:

Food dyes are compounds that are widely used in the food products industry, as they give distinctive colors to food products that make them more attractive to the consumer. **(Alessandra Durazzo,2022)** In this work, we studied and estimated a group of these food dyes are (E102- Tartrazine) a lemon-colored yellow pigment, (E110- Sunset yellow) a yellow orange pigment and (E122-Azorubine) a red pigment. These dyes are characterized by their high-water solubility (**Barciela,2023**) and have an absorbing spectrum within the visible and ultraviolet spectrum based on the pH of the solution they contain. For example, E110- Sunset yellow has maximum absorption at about 480 nm at pH 1 and 443 nm at pH 13 with a shoulder at 500 nm (**Liudi Ji 2016**). Given the high demand for soft drinks in all countries of the world, processed food dyes are of great importance in this regard, it offers many advantages that are not found in natural dietary dyes in terms of their chemical composition stability over a wide thermal range are unaffected by biological contaminants, persistent over a long storage period and not affected by atmospheric oxygen (**Newsha Mahmoodi 2016**). It also has a high value Absorptivity Coefficient (ε) and therefore the color will be deep and wide using the lowest concentrations. The reason for this is the existence of successive double bonds such as the azo group and the aromatic rings. Because these manufactured food dyes are chemical compounds and contain a colored azo group as well as organic aromatic rings, they are not without health risks for those who consume these soft drinks (**Barciela,2023**). Various governments have therefore introduced regulations and legislation that determine the type and quantity of dyes that are allowed to be added to different foods and food products in the UE (**directive 94/36/1994**). These regulations vary from one country to another because excessive consumption of these dyes can cause diseases (**Ou Sha 2014**, **Kamel M. M. 2011, W. Zhang 2009**). Hence, the development of methods of estimating these food dyes found in food products is very important, preferably more accurate, cheaper, and have faster results. It should be noted here that chromatographic methods are characterized by their ability to separate sample ingredients from the food dyes to be estimated, and therefore by measurements of the standard solutions for these dyes. In some cases, the estimation requires preliminary operations, represented by the extraction of these dyes from the samples in which the dyes are to be estimated. The extraction processes are accurate, sensitive, and reliable to ensure an accurate estimation of these dyes in the samples studied (**N. Pourreza, 2011, M. S. El-Shahawi, 2013**). Below are the different methods and techniques that have been used in the quantification and analysis of these dyes in food products mentioned in the literature. Some of these methods require a preliminary extraction process and an preconcentrating of the dye before starting to measure it. There are several methods to estimate these compounds, such as Spectrophotometric method (**N. E. Llamas, 2009**) and liquid chromatography procedure, (**Yahya S. Al-Degs 2009**,), electrochemical methods (**Mingyong Chao 2014**), high performance liquid chromatography procedure**,(M. G. Kiseleva,2003**, **Behrouz Akbari-Adergani 2018, M.S García-Falcón 2005**) and other methods. The European Union has set standards and specifications for the use of these food dyes in food products, including a comprehensive and regulatory vision for the use of these dyes in food products. The permissible levels in non-alcoholic drinks, flavourings, sweets and luxury baked goods have been determined. Colors may be used up to the appropriate limit indicated in the tables, but the amounts of E 110 and E 122 may not exceed 50 mg/kg or mg/L (**directive 94/36/1994**) ... A guide has also been organized for chemical analysts working in the estimation of these dyes in food products and the maximum permissible concentration of these food dyes (alone or in combination).   All these legislations, regulations and specifications have been developed for the purpose of consuming food products safely for the consumer.

Experimental

Equipment:

In this research was used a Shimadzu HPLC with a low-pressure gradient system, connected to a manual injection valve. A 20 µl internal sample loop was used to inject the sample. The sample was separated By using A NUCLEODUR® 100-5 C18 ec (250 × 4.6 mm i.d.) (MACHEREYNAGEL Germany) stainless steel, reversed phase column, with 5-µm particle size, A REF 718966 guard column holder and an EC guard column cartridge (4 mm × 3 mm i.d.). The Components are detected at a wavelength of 374 nm. The system contains a CTO-20A vertical oven, with forced air circulation. It can regulate the temperature from 10°C above room temperature to 85°C. Rapid temperature stabilization allows for complex temperature increases and programs including linearity and temperature decreases. The effect of temperature on separation efficiency can be studied.

Chemicals & Reagents:

Standard food dyes were obtained from the local market. Juices and soft drinks were brought from the local market from different locations and contain various food dyes. The chemicals and solvents used in this study were of HPLC grade.

Preparing standard solutions:

Standard solutions for these three dyes were prepared with a concentration of 500 ppm, (stock solution) a volume of 500 ml, and a weight of 0.25 g they were dissolved in deionized water. After that, solutions were prepared at a concentration of 10 ppm of the original solution by dilution, and they were used in subsequent experiments.

Determination the wavelength:

Including studying the absorption spectra within the visible and ultraviolet (UV-Visible) region each pigment separately, and then the spectra were added to each other. The spectrum in Figure 1 below. From the absorption spectrum of the three dyes, the wavelength 473 nm was chosen as a common wavelength, and the wavelength of the HPLC detector was adjusted to be able to detect the three dyes as they passed through. On the other hand at the 234 nm we can uses to common wave length and its more sensitive but in these region its overlap with absorbance of the solvent.

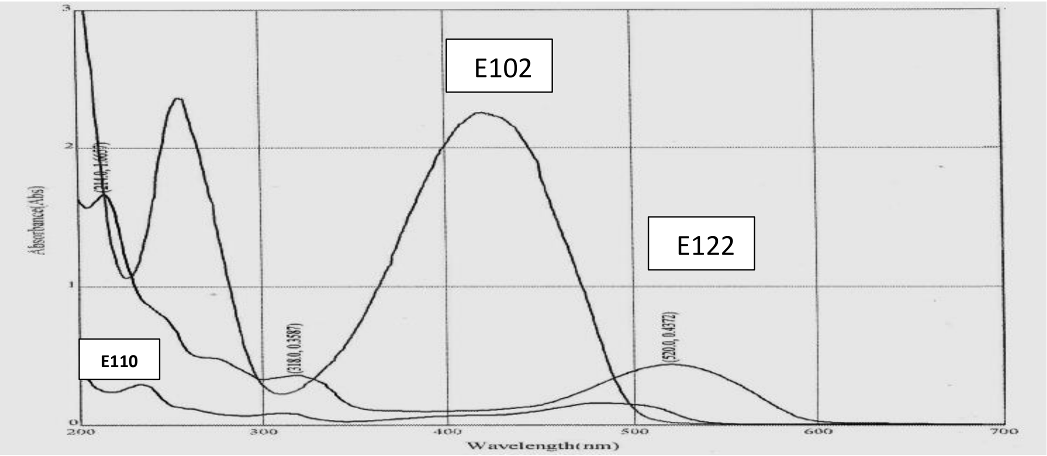

Figure (1): The absorption spectra of the three dyes at 10 ppm conc.

Results and discussion:

In this research related to the Determination of the three food dyes mentioned above, preliminary tests were performed on the mixture of the three dyes at a common wavelength of 473 nm, a mobile phase speed of 1 ml/min, and a mobile phase mixture of (75:25 - deionized water: methanol) the results are shown in Figure (2) below.

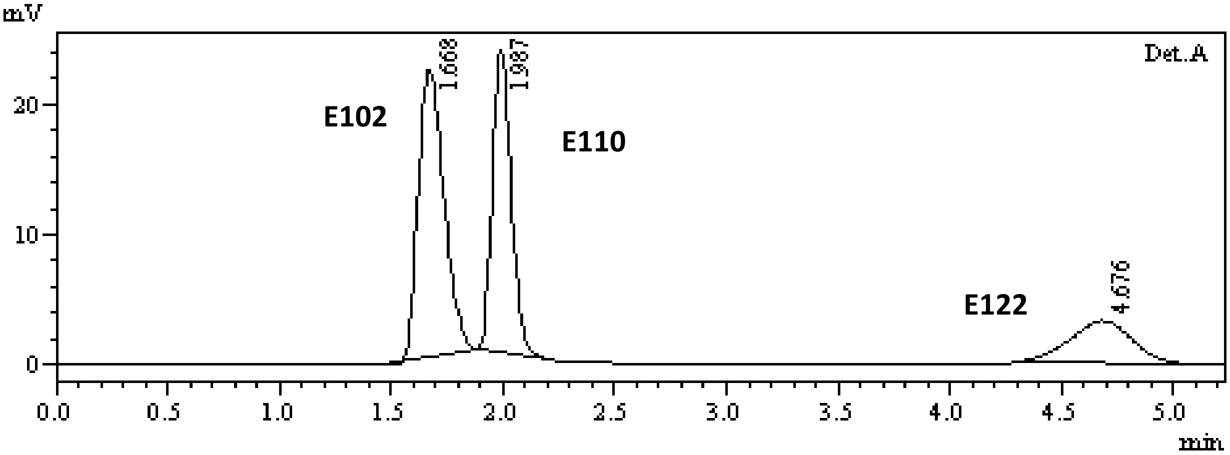

Figure (2): chart for the three food dyes on preliminary tests.

After carrying out the initial tests, it was the turn to study the influence of the factors affecting the separation process, including the strength of the mobile phase, temperature, and flow rate of the mobile phase, in order to reach the optimal conditions for the separation process and determine them in terms of time and cost with precision and accuracy.

Effect of mobile phase composition:

The effect of changing the mixing ratios of the mobile phase components on the separation efficiency and retention time of this food dyes was study. isocratic elution (methanol, deionized water), was used in the mobile phase, the flow rate (1 mL/min). the sample consisting of mixture of the three dyes under study. It turns out that changing the proportions of the mobile phase greatly affects the retention time and separation time values, and this indicates that changing the polarity of the mobile phase affects the interaction process between the mobile phase and the components of the sample. It also indicates that these dyes are characterized by their relative polarity, which makes them affected by the polarity of the mobile phase because we know that changing the proportions of the mobile phase affects its polarity. In the table below, the values of the proportions of the mobile phase, retention times, Dye, and type of dye. The results are shown in Table (1) and Figures (2-3-4) below.

Table (1): the values of tR, k and N of each Dye at different percent organic modifier

Mobile phase (v/v) deionized water: methanol	**Dyes**	**Parameter**		
		t_R	**k**	**N**
75:25	**E 102**	**1.668**	**1.38**	**495**
	E 110	**1.987**	**1.84**	**702**
	E 122	**4.676**	**5.68**	**714**
76:24	**E 102**	**1.668**	**1.38**	**495**
	E 110	**1.991**	**1.84**	**705**
	E 122	**4.986**	**6.12**	**551**
77:23	**E 102**	**1.678**	**1.40**	**368**
	E 110	**1.999**	**1.86**	**710**
	E 122	**5.424**	**6.75**	**735**
78:22	**E 102**	**1.680**	**1.40**	**502**
	E 110	**2.114**	**2.03**	**799**
	E 122	**5.771**	**7.26**	**660**
79:21	**E 102**	**1.689**	**1.41**	**285**
	E 110	**2.176**	**2.11**	**842**
	E 122	**7.940**	**10.34**	**1009**
80:20	**E 102**	**1.691**	**1.42**	**286**
	E 110	**2.272**	**2.25**	**516**
	E 122	**9.240**	**12.20**	**1033**

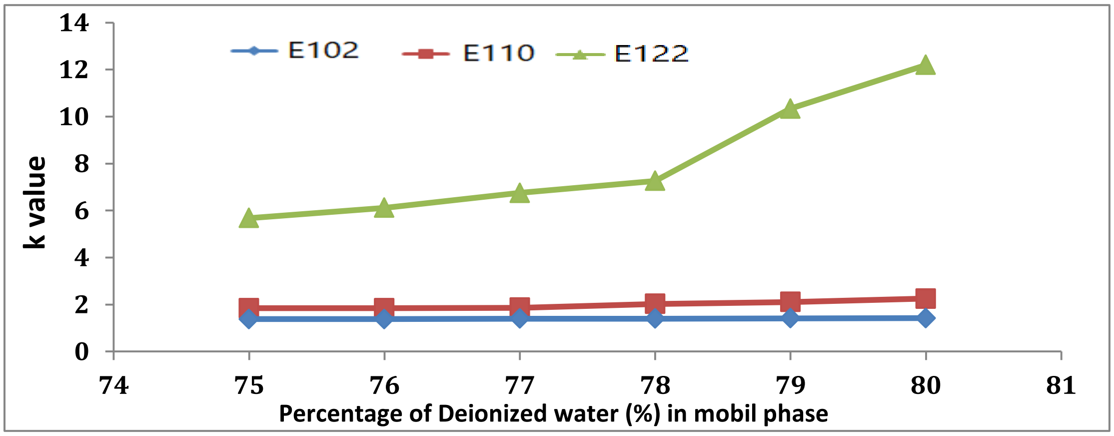

Figure (3): the effect of organic modifier percentage on the k value.

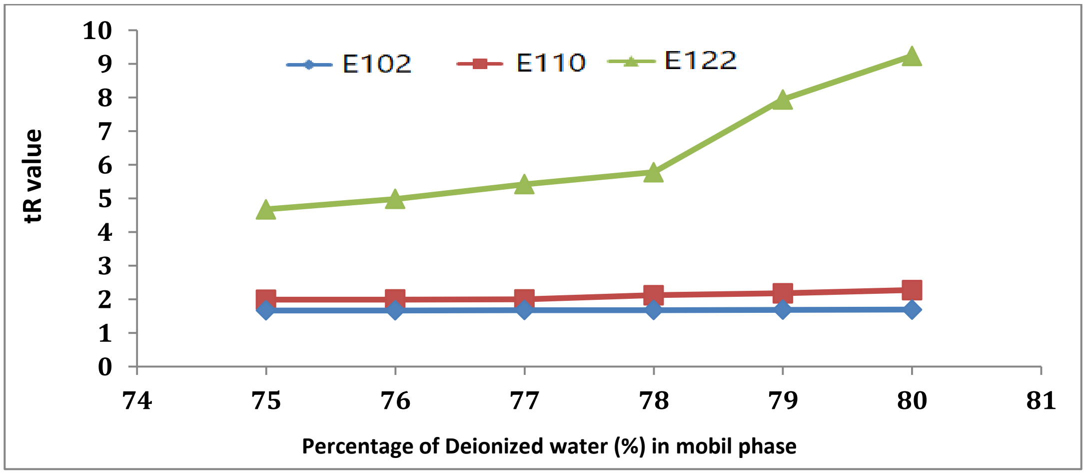

Figure (4): Effect of organic modifier percentage on the tR value.

From the results and figures, was note that the ratio (78:22 - deionized water: methanol) is considered the best composition ratio for the mobile phase because it achieves a short (analysis time) with good separation. On this basis, this ratio was used in the subsequent stages of this research. In the figure (4) below is the analysis chart for the three food dyes under study.

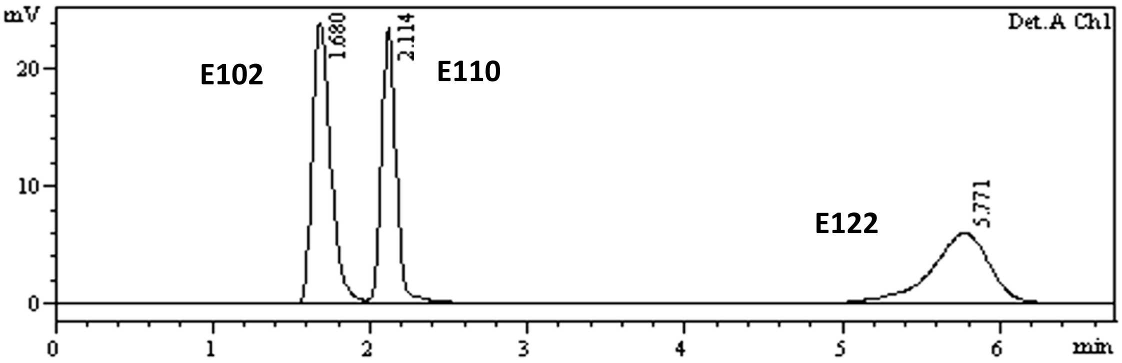

Figure (5): chart for the three food dyes on best composition ratio (78:22).

Effect of changing the flow rate:

The effect of changing the mobile phase flow rate was studied to determine the optimal flow rate within the range (0.7-1.2 ml/min) to obtain the best separation speed while ensuring that the pressure did not rise so as not to affect the pump life. The mobile phase flow rate (1 mL/min) was chosen as the optimal speed giving a good separation and short analysis time with reasonable pump pressure.

Table (2): Result of tR, k, α and Rs of three dyes at different flow rates.

F. R. (mL/min)	Dyes	tR	k	α	Rs
0.7	E 102	2.32	3.73	1.27	1.32
	E 110	2.81	4.73	------------	---------
	E 122	8.47	16.29	3.44	8.58
0.8	E 102	2.04	2.66	1.29	1.23
	E 110	2.48	3.43	------------	---------
	E 122	7.50	12.41	3.62	7.74
0.9	E 102	1.84	1.92	1.32	1.11
	E 110	2.23	2.54	------------	---------
	E 122	6.48	9.29	3.66	6.80
1	E 102	1.68	1.40	1.45	1.60
	E 110	2.12	2.03	------------	---------
	E 122	5.78	7.26	3.85	7.71
1.1	E 102	1.52	0.97	1.45	1.31
	E 110	1.86	1.42	------------	---------

	E 122	5.66	6.35	4.49	7.45
1.2	E 102	1.42	0.69	1.52	1.00
	E 110	1.72	1.05	------------	---------
	E 122	5.30	5.31	5.07	7.16

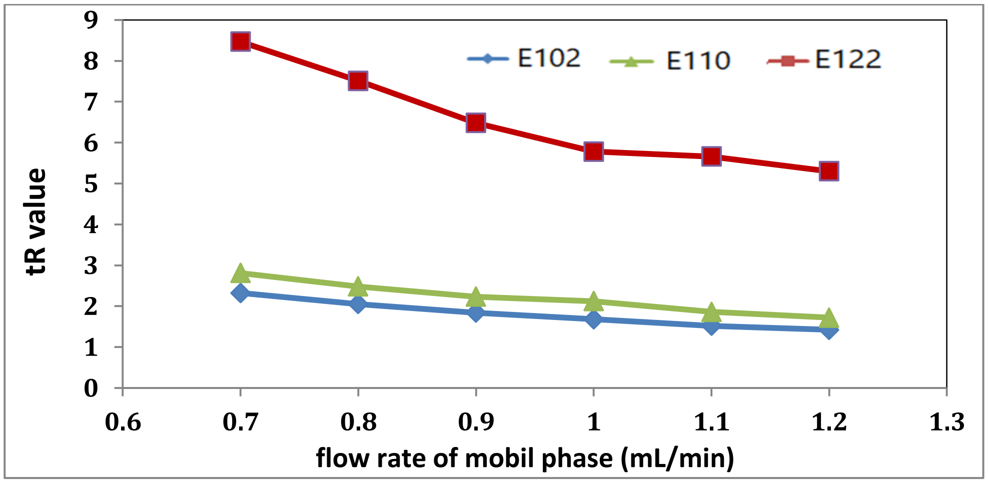

Figure (6): effect of flow rate on the tR.

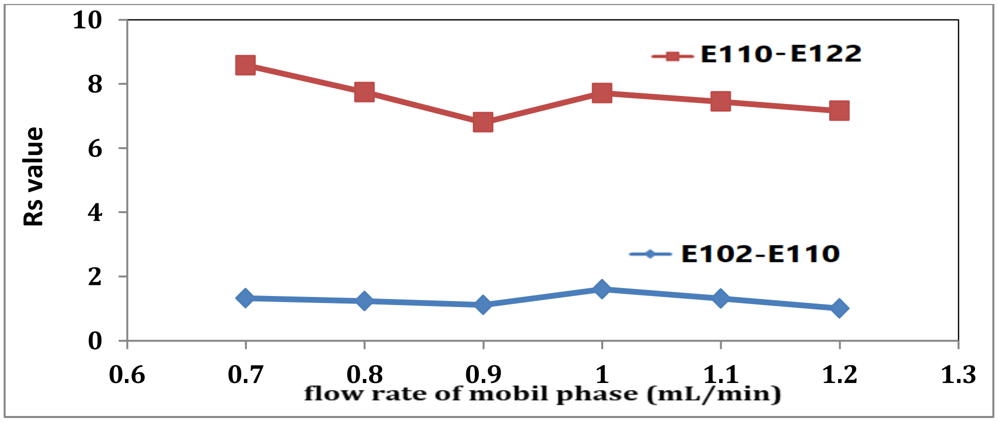

Figure (7): effect of flow rate on the Rs.

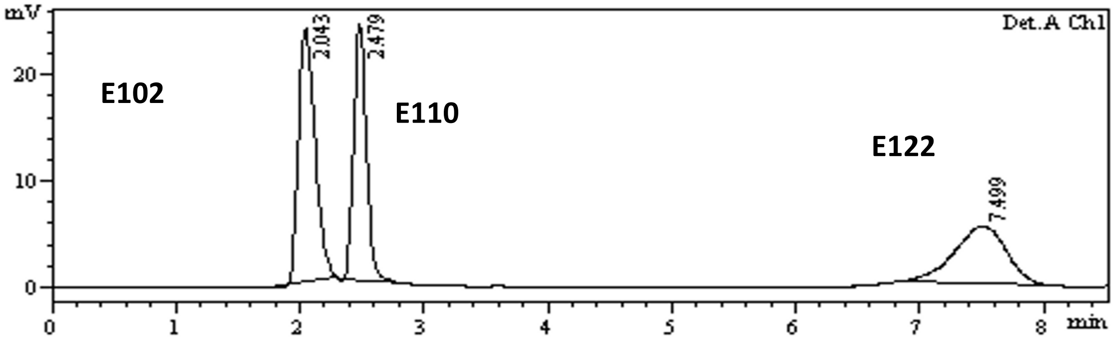

Figure (8): chart for the three food dyes at the best flow rate 0.8 mL/min.

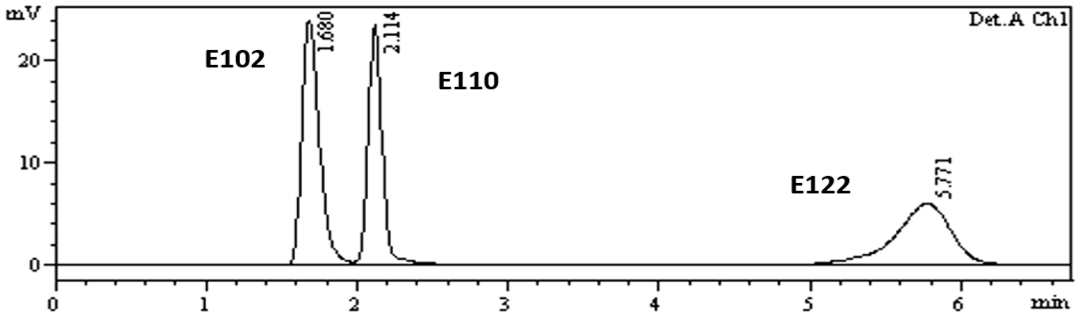

Figure (9): chart for the three food dyes at the best flow rate 1 mL/min.

Effect of temperature on separation parameters:

The effect of changing the oven temperature at the thermal range (30, 35, 40, 45, 50, 55, and 60 degrees Celsius) was studied by controlling the convection oven temperature. It was observed that increasing the column temperature in RP-HPLC leads to a change in retention times and efficiency column due to the change in viscosity of the mobile phase with change in temperature [**Hadi Hassan Jasim 2017**]. Below is a table with the values of the factors affecting the separation efficiency, along with some illustrative figures. Through studying the effect of temperature change, the degree (45°C) was chosen to be optimum temperature its giving a good separation and short analysis time at (22:78 - methanol: deionized water) with 1 mL/min flow rate.

Table (3): Result of tR, k , N, α, and Rs three dyes at changing the column temp.

Temp ^{0}C	Dyes	tR	k	α	Rs
35	E 102	1.71	1.44	1.74	1.36
	E 110	2.46	2.51	------------	------------
	E 122	10.1	13.43	5.34	7.84
40	E 102	1.69	1.41	1.52	1.36
	E 110	2.2	2.14	------------	------------
	E 122	7.67	9.96	4.65	6.84
45	E 102	1.68	1.40	1.44	1.43
	E 110	2.11	2.01	------------	------------
	E 122	5.77	7.24	3.60	5.86
50	E 102	1.67	1.39	1.40	1.42
	E 110	2.06	1.94	------------	------------
	E 122	4.80	5.86	3.01	5.77

55	E 102	1.65	1.36	1.33	1.00
	E 110	1.96	1.80	------------	------------
	E 122	4.07	4.81	2.67	4.59
60	E 102	1.64	1.34	1.27	0.91
	E 110	1.89	1.70	------------	------------
	E 122	3.77	4.39	2.58	5.61

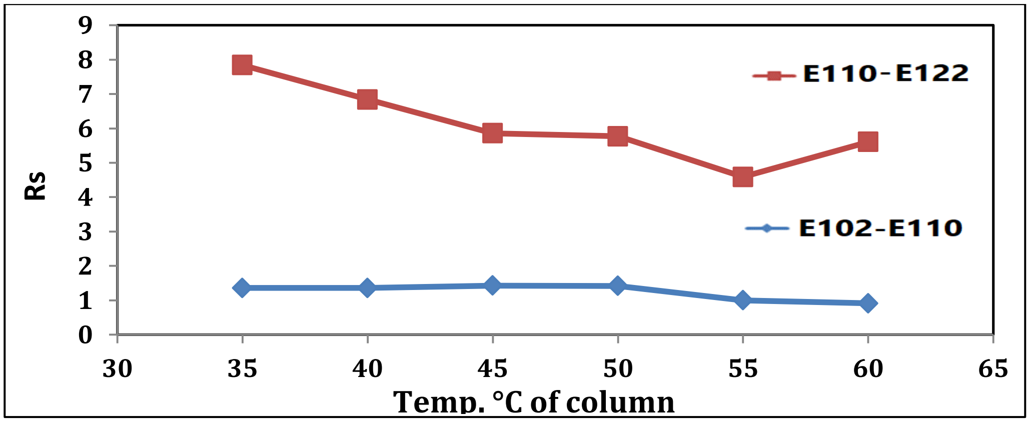

Figure (10): Effect of column temperature on the RS value

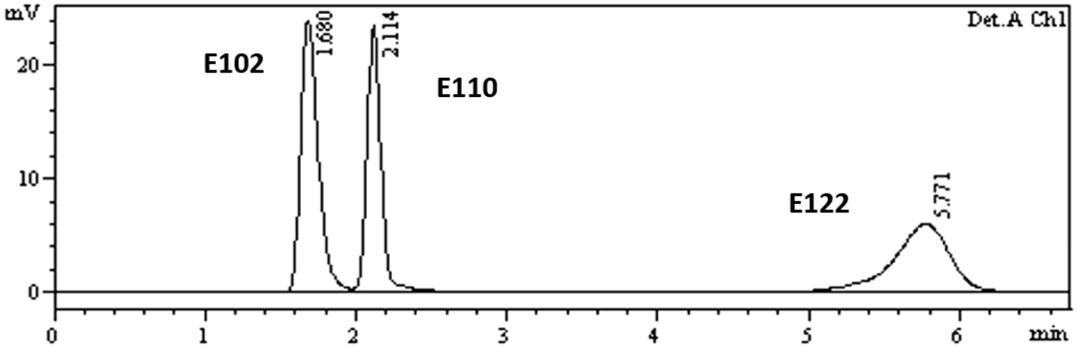

Figure (11): Chromatograms of three food dyes at 45 ^{0}C.

Separation process by using the gradient elution system.

All the experiments that were conducted previously were on the Isocratic elution system, and in this paragraph we are doing a study on the separation of the three food dyes according to the gradient elution system In this type of separation system, we can control the mixing ratios in the mobile phase and the wavelength of the detector during the run, and through it we can obtain an good separation with high efficiency, , and a short time with consistent peaks, so the Gradient system was created for the separation of The three foods dyes Under study to obtaining good results, it can be used to estimate and separate these dyes when studying samples containing these food dyes. Below is a table (4) common wavelength of the values of the variables that were used in this system, with illustrative figures. flow rate (1 mL/min), temp. 45C^0.

Table (4): **Gradient elution program for separation of three foods dyes.**

Time(min)	Module	Action	Value
0.10	Pumps	Deionized water %	88%
0.10	Pumps	Methanol %	12%
0.50	Pumps	Deionized water %	80 %
0.50	Pumps	Methanol %	20 %
1.10	Pumps	Deionized water %	70 %
1.10	Pumps	Methanol %	30 %
10.00	Controller	Stop	

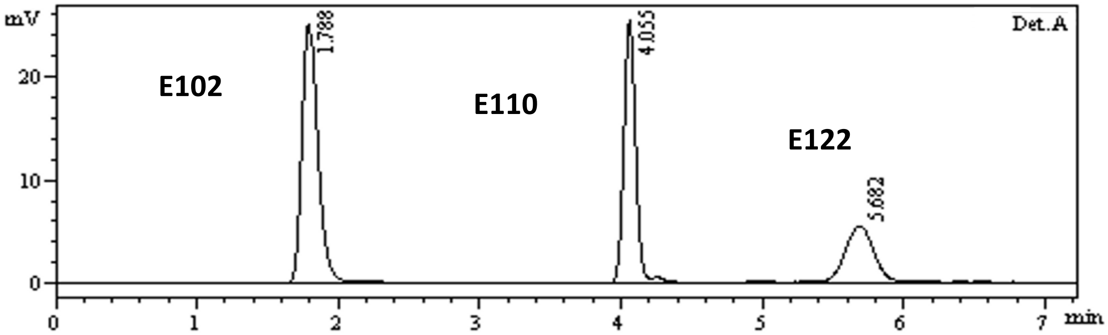

Figure (12): Chromatograms of three food dyes at gradient elution

Calibration curve:

In this study, the calibration curve for the three food dyes was drawn at the concentration range of (1-25 µg/mL) from the peak area of the two dyes (E102 and E122) and for (E110 dye) the concentration range of (1-30 µg/mL) from the peak area. The linearity, curve equation and slope were extracted by linear regression equation as shown in the figure and table below.

Table (5). Calibration curve results

Dyes	Linearity range (µg/mL)	Calibration graph	R^2	Slop	Intercept	LOD (µg/mL)	LOQ (µg/mL)
E 102	**1-25**	**Peak area**	**0.9983**	**56470 (µv.mL.s.µg-^{1})**	**-34914 (µv.s)**	**0.905**	**2.743**
E 110	**1-30**	**Peak area**	**0.9972**	**5789.8 (µv.mL.s.µg-^{1})**	**- 4231.3 (µv.s)**	**0.124**	**0.377**
E 122	**1-25**	**Peak area**	**0.9919**	**26461 (µv.mL.s.µg-^{1})**	**-517 (µv.s)**	**2.260**	**6.848**

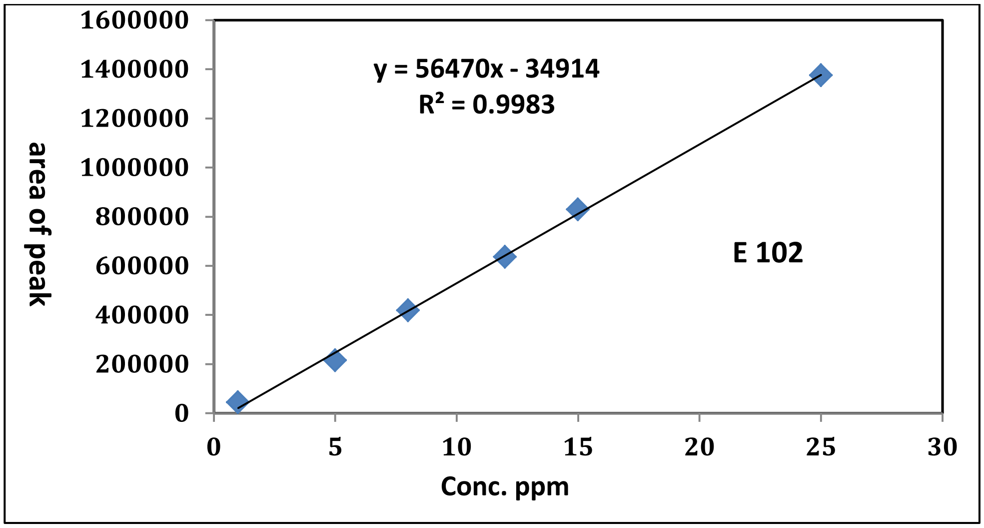

Figure (13): Calibration curve of E 102.

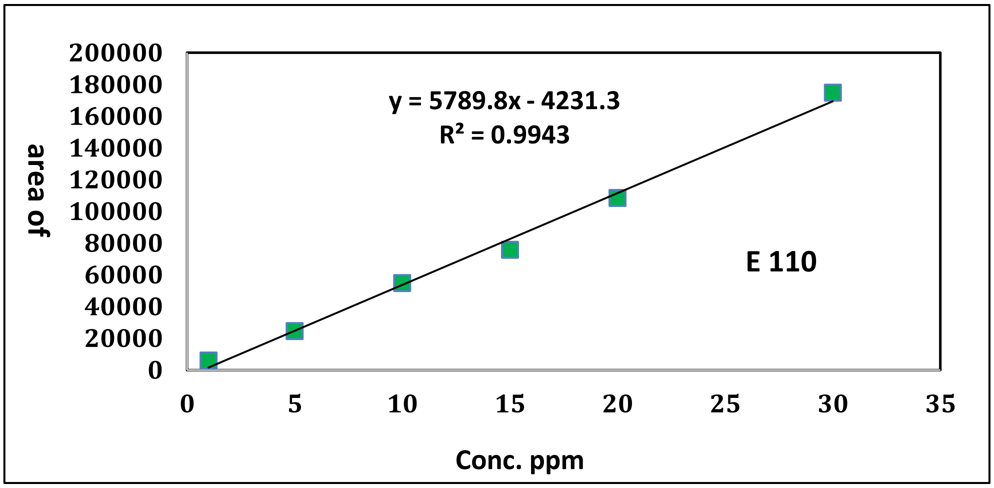

Figure (14): Calibration curve of E 110.

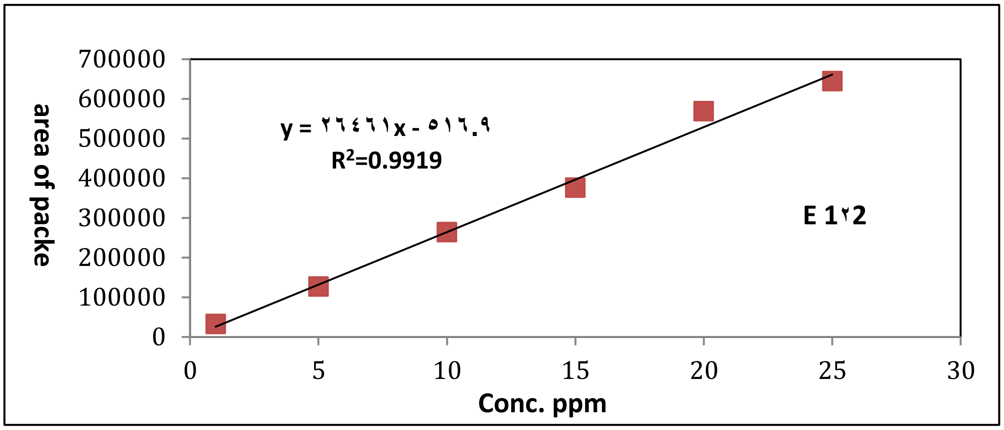

Figure (15): Calibration curve of E 122.

The method validation:

In this study, the accuracy (Recovery or Er%) and precision (SD or RSD%) of this method were calculated to determine its suitability for application in the separation and estimation the three food dyes at above. in the table below are values for these factors determining the suitability of the method. The recovery in range of (88.4 – 102) and the RSD (0.14 – 5.33) %.

Table (6): the accuracy and precision of this method.

Dyes	Conc. Taken (µg / mL)	Conc. Found* (µg / mL)	Recovery	RSD%
E 102	**5**	**4.42**	**88.4**	**0.14**
	15	**15.30**	**102**	**5.33**
E 110	**5**	**4.93**	**98.6**	**4.64**
	15	**13.79**	**91.9**	**2.3**
E 122	**5**	**4.8**	**96.0**	**3.46**
	15	**14.2**	**94.66**	**1.14**

*Average of three readings.

The Application of the method: -

The contents of E102, E110 and E122 were misheard and determined in soft drink from local market. The results are shown in Table (7). The RSD% ranged between 0.69 - 5.20 %.

Table (7). the amount of food dyes present in soft drinks.

Name of soft drink	Name of food dye	Conc. Found (μg / mL)	RSD%
Retoosh	**E 102**	**15.89**	**2.43**
Dimond	**E 110**	**14.96**	**5.20**
Tazij	**E 122**	**39.21**	**0.69**

Conclusion:

In this research, a quick, easy, and accurate method was found to evaluate and estimate the concentrations of food dyes used in food products. This method can be used in laboratories to monitor the quality of food products to limit the excessive use of these dyes and limit their impact on the health of individuals and people who eat these food products to protect them and maintain their health, within the limits of concentrations allowed by the World Health Organization and similar organizations in countries around the world.

References:

Alessandra Durazzo, Márcio Carocho , and others, "Food dyes and health: Literature quantitative research analysis" Measurement: Food Volume 7, September 2022.

Akbari-Adergani B, Poorasad M., Esfandiari Z., "Sunset yellow, tartrazine and sodium benzoate in orange juice distributed in Iranian market and subsequent exposure assessment"International Food Research Journal January 2018, 25(3):975-981.

Barciela, Paula, Ana Pérez Vázquez, M.A. Prieto, "Azo dyes in the food industry: Features, classification, toxicity, alternatives, and regulation. *Food and Chemical Toxicology*" Volume 178, August 2023.

Hadi Hassan Jasim, Sarmad Bahjat Dikran, Amer Saleh Mahdi, Bahaa Malik Altahir Determination of insecticides in different commercial formulations by Gradient HPLC’ *Br. J. Anal. Chem.,* 2017, *4* (14), pp 25 – 33.

Liudi Ji, Qin Cheng, Kangbing Wu. "Cu-BTC frameworks-based electrochemical sensing platform for rapid and simple determination of Sunset yellow and Tartrazine" *Sensors and Actuators B: Chemical* Volume 231, August 2016, Pages 12-17.

M. M. Kamel and S. H. El-lethey, “The potential health hazard of tartrazine and levels of hyperactivity, anxiety-like symptoms, depression and anti-social behaviour in rats,” *Journal of American Science*, vol. 7, pp. 1211–1218, 2011.

M. G. Kiseleva, V. V. Pimenova, and K. I. Eller "Optimization of Conditions for the HPLC Determination of Synthetic Dyes in Food "Journal of Analytical Chemistry, Vol. 58, No. 7, 2003, pp. 685–690.

Mingyong Chao, Xinying Ma "Convenient Electrochemical Determination of Sunset Yellow and Tartrazine in Food Samples Using a Poly(L-Phenylalanine)-Modified Glassy Carbon Electrode " Food Analytical Methods April 2014, 8(1):130-138.

M.S. García-Falcón, J. Simal-Gándara "Determination of food dyes in soft drinks containing natural pigments by liquid chromatography with minimal clean-up" Food Control Volume 16, Issue 3, March 2005, Pages 293-297.

M. S. El-Shahawi, A. Hamza, A. A. Al-Sibaai, A. S. Bashammakh, and H. M. Al-Saidi, “A new method for analysis of sunset yellow in food samples based on cloud point extraction prior to spectrophotometric determination,” *Journal of Industrial and Engineering Chemistry*, vol. 19, no. 2, pp. 529–535, 2013.

N. E. Llamas, M. Garrido, M. S. D. Nezio, and B. S. F. Band, “Second order advantage in the determination of amaranth, sunset yellow FCF and tartrazine by UV-vis and multivariate curve resolution-alternating least squares,” *Analytica Chimica Acta*, vol. 655, no. 1-2, pp. 38–42, 2009.

N. Pourreza and M. Ghomi, “Simultaneous cloud point extraction and spectrophotometric determination of carmoisine and brilliant blue FCF in food samples,” *Talanta*, vol. 84, no. 1, pp. 240–243, 2011.

Newsha Mahmoodi, Mohammad Faraji, Parisa Ziarati. "Simultaneous Determination of Sunset Yellow and Carmoisine in Orange Flavored Soft Drink Samples by High-Performance Liquid Chromatography" *Journal of Applied Chemistry*, 9(08) page 79-83, (2016).

Ou Sha, Xiashi Zhu, Yanli Feng, Weixing Ma, "Determination of Sunset Yellow and Tartrazine in Food Samples by Combining Ionic Liquid-Based Aqueous Two-Phase System with High Performance Liquid Chromatography", *Journal of Analytical Methods in Chemistry*, vol. 2014, Article ID 964273, 8 pages, 2014.

W. Zhang, T. Liu, X. Zheng, W. Huang and C. Wan, "Surface-enhanced oxidation and detection of Sunset yellow and Tartrazine using multi-walled carbon nanotubes film-modified electrode" Colloids and Surface B, 74, 2009, 28–31.

Yahya S. Al-Degs . "Determination of three dyes in commercial soft drinks using HLA/GO and liquid chromatography" *Food Chemistry* Volume 117, Issue 3, 1 December 2009, Pages 485-490