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Analytical survey with HPLC-DAD of synthetics food dyes in liquid matrix frequently used in Morocco.

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ABSTRACT

The quantification method of 11 foods dyes offers an excellent combination of sensitivity, selectivity, simplicity and analysis time (45 min) with relatively short quantification limits respectively of 0.03 ppm and 0.09 ppm order. She obtained by Canadian method of validation. Compared to the spectral technique; this method allows the separation and quantification at very low concentrations mixtures of more than two colors. While the spectral technique is incapable. Compared with thin layer chromatography, which is a qualitative technique, in addition to identifying coloring, it allows the spectral identification and quantification for dyes present in various foodstuffs.

Keywords: Food analysis, Synthetic dyes, Liquid chromatography, Food additives, HPLC/DAD.

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INTRODUCTION

The food colorants (natural or synthetic) are added to foodstuffs in order to make them more visually attractive to consumers and to restore their original appearance when it has been lost during production processes. They are extensively used to offset the loss of natural food colors that are destroyed during processing and storage and to provide the desired color appearance. Synthetic dyes are a very important class of food additives [1]. European Directive 1994/36/EC [2] lists the colors that can be added to food: it also defines foodstuffs to which only certain colorants may be added, their permitted maximum level and their use restrictions as well. In fact, the permitted quantity of synthetic dyes is strictly regulated because of their potential risk to human health.

In general, "Analytical Chemistry seeks ever improved means of measuring the chemical composition of natural or artificial materials" and the Analytical Chemists is the person that works to improve the reliability of existing techniques to meet the demands for better chemical measurements" [3]

The contribution of analytical chemistry in the determination of different synthetic food dyes is of great importance, given the large number of technical deployment, such as quantification by UV- Visible but it is limited to a matrix with a mixture of three dyes maximum [4]. There are also methods include techniques such as thin layer chromatography (TLC) [5,6] , high performance TLC combined with image processing [7] , solid phase spectrophotometry [2,8,9] , adsorption voltammetry [11] , but these methods have a pretreatment time of the relatively long sample . Analysis based on liquid chromatography spectrometry methods [12-14] are particularly suitable for the analysis of toxic and illegal dyes because they have been developed to unambiguously identify dyes trace. The objective of the present work is to develop an HPLC method coupled with a diode array detector (DAD) for the determination of synthetic dyes in different types of food and beverages.

The analyzed dyes in this study, were the azo-compounds tartrazine (E102), sunset yellow (E110), azorubine (E122), amaranth (E123), allura red (E129),Red ponceau 4R (E124) ,quinophthalone quinoline yellow (E104), triarylmethane compounds patent blue V (E131) and brilliant blue FCF (E133) , the indigo colorant indigo carmine (E132) and the Xanthène compounds the Erythrosine (E127).(Fig1)

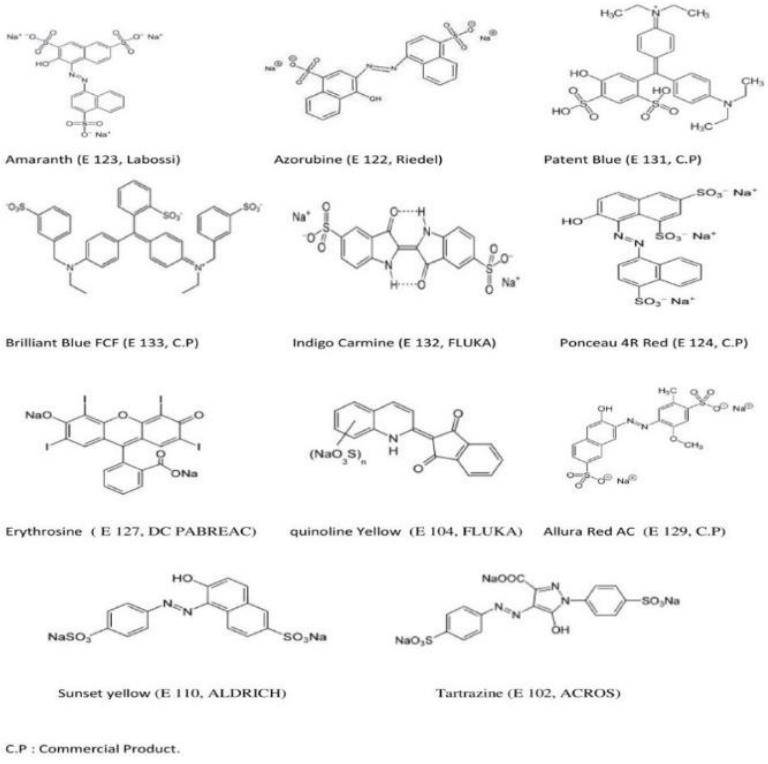

Figure 1: Chemical structures, common names, E (European Community) and brands of synthetic food colorants studied.

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The method [15] with the Both isocratic [16,17,18] and gradient [19,20,21,22] systems are used, and the last are preferred for the separation of the more complex mixtures. It was validated according to DR-12-VMC Edition 2009 memorandum for the validation method in chemistry [10] based on the international standard ISO / IEC 17025 for the detection and quantification of all dyes studied in beverages and solid food matrices.

MATERIALS AND METHODS

Reagents

Ultrapure Water Aurora Crysta was used for the deionized water to prepare all the solution and all chemicals were of analytical grade, unless otherwise-against. All chemical used (Table. 1) were of analytical grade.

Table 1: Chemicals used and their brands.

t (min)	A (%)	B (%)
0	100	0
2	100	0
22	47.5	52.5
37.6	0	100
40	0	100
41	100	0
43	100	0

Apparatus

Chromatographic analyzes were performed with the liquid chromatograph Agilent 1100 Series HPLC equipped with a quaternary gradient pump Agilent 1200 Series capable of mixing four solvents. A series of sample Autosampler Agilent system 1100, a manual injector (MI) 100 µL of detector and a variable wavelength detector G1314A (VWD) with standard flow cell (10 mm path length, 14 µl volume, maximum pressure 40 bar) diode array. The chromatographic data were collected and processed using a personal computer running Agilent ChemStation.

A pH meter equipped with a combined glass calomel electrode was used for pH measurements. The determination of the purity of the 11 dyes (fig 1) was performed with a double beam spectrophotometer UV-1601 UV / Vis SHIMADZU AX200 with 1 cm quartz cells (Shimadzu).

Development of standards for paint and sample solutions

Standard solutions containing 100 mL of each dye were prepared with 1000 ppm of pure dye in deionized demineralized water. The solutions were maintained in flasks. The working standards of each color solutions were prepared by appropriate dilution of the stock solutions with deionized water to give concentrations of between 0.10 and 50 mg.L-1 (PPM). Mixed standard solutions containing all dyes in concentrations between 0.10 and 10 mg.L- 1 were also prepared by mixing and diluting the appropriate aliquots of each standard substance solution. All solutions were stored at 3◦C in the dark and are stable for at least 3 months.

Chromatographic conditions

A column (250 mm x 4.6 mm) fully end-capped with 5- spherical particles _M and with a load of 12 % of carbon (3 µmol m-2) was used with C18 (25 mm × 4.6 mm , 5 µm) guard column (Supelco) . AC18 column is the type of column used as laboratories for routine analysis.

The mobile phase is an aqueous ammonium acetate solution solution1 % (m / v) at pH 7.5 by adding a few drops of a sodium hydroxide solution 10% (m / v) (mobile phase A) and a mixture of methanol :

acetonitrile (80:20 v / v) (mobile phase B) . The mobile phase A was filtered by suction through a membrane filter with a pore diameter of 0.45 µm.

The eluent flow rate was kept constant at 1.5 mL min-1 and the injection volume was set at 20 µL . The gradient program used [15], is given in Table.2. All experiments were performed at room temperature.

The diode array detector is programmed to monitor the dyes on the next wave length: 435 (yellow), 530 (red) and 620 nm (blue). The chromatographic system was initially conditioned by passing the mobile phase through the column until a signal A of stable base line was obtained.

Product	*Marque*
Ammonium acetate	FLUKA
sodium hydroxide (NaOH)	SIGMA-ALDRICH
methanol (MeOH) [HPLCgrade]	CARLO EBRA SDS
acetonitrile (ACN) [HPLCgrade]	CARLO EBRA SDS

Table 2: Gradient program used for analysis of the 11 dyes by HPLC-DAD.

Determination of the purity of the dye

Purities of the dyes were determined by measuring based on the method of spectrophotometric absorbance diluted standard solution (10 mg L-1), for which the Beer-Lambert law is valid. The percent amount of dye purity is given by equation [15]: % Purity = DF × A / (A1%1 cm × C) × 105 (1).

Where DF is the dilution factor of the measured from the stock solution of the standard solution, A the absorbance of the test sample (A <1) relative to the water, the expression A1% 1 cm is the specific absorbance of a 1% (10 g L-1). An aqueous solution of the dye to the wavelength prescribed in using a path length of 1 cm at 20 ± 1◦C, and this is the concentration of the mother solution expressed as mg of the unpurified coloring matter in 100 mL of distilled deionized water. The A1%1cm value for each compound is presented in Table.3 [15].

Dye	*A1%1 cm*	*Purety %*
Amaranth	440	76.33
Azorubine	510	74.62
Brilliant Blue FCF	1630	57.38
Patent Blue	2000	54.96
Erythrosine	1100	76.94
Indigo Carmine	480	83.52
Quinoline Yellow	865	72.26
Sunset Yellow	555	77.90
Allura Red AC	540	81.81
Ponceau Red	430	63.21
tartrazine	430	80.57

Table 3: Calculated purities , A1%1 cm, specific absorbance and of synthetic colorants studied.

RESULTS AND DISCUSSION

Separation

We got through the gradient (Tab.2) as a good separation of 11 colors (Fig.2) with the quinoline yellow that gives three pikes, check with several injection of the dye to ensure comparability of these spades this dye. probably as a separation of the product, the superposition of the peaks of Brilliant blue FCF and Azorubine gives no interference for each dye adsorbed in different range. All that this does not affect the identification and quantification.

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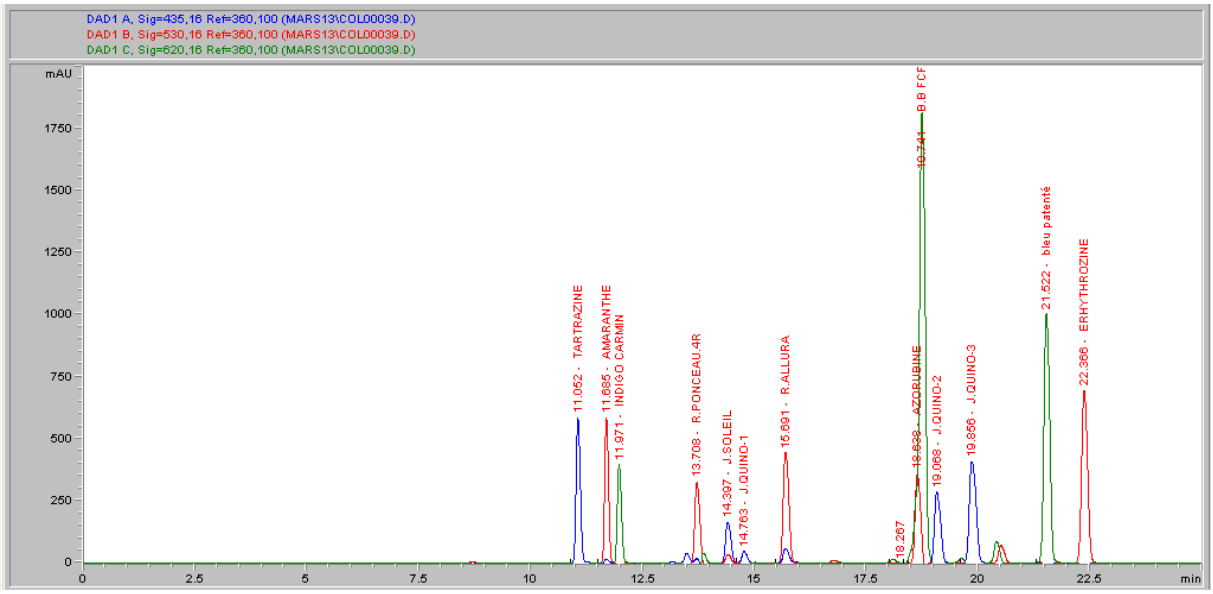

Figure 2: Chromatogram of a mixed standard solution of 11 dyes.

Method validation

Method validation is the process used to confirm that the analytical procedure employed for a specific analysis is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

Analytical methods need to be validated or revalidated before their introduction into routine use; whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and whenever the method is changed and the change is outside the original scope of the method.

Limit of detection of method (LOD)

The limit of detection is a method for the lowest concentration tested in a real matrix that, when subjected to all the steps of a complete method, including chemical extraction and pretreatment compound, produces a detectable signal with a reliability defined statistically different from that produced by a "Blanck" under the same conditions.

Dye	*standard deviation (ppm)*	*LOD valued (ppm)*	*5 x LOD V (ppm)*	*7 x LOD V (ppm)*
Amaranth	0.020	0.061	0.308	0.432
Azorubine	0.026	0.078	0.394	0.551
Brilliant Blue FCF	0.006	0.018	0.094	0.132
Patent Blue	0.012	0.038	0.192	0.269
Erythrosine	0.016	0.048	0.240	0.336
Indigo Carmine	0.012	0.037	0.187	0.262
Quinoline Yellow	0.011	0.033	0.168	0.236
Sunset Yellow	0.057	0.171	0.855	1.197
Allura Red AC	0.020	0.061	0.308	0.432
Ponceau Red	0.108	0.325	1.627	2.277
Tartrazine	0.041	0.125	0.627	0.878

Table 4: **Standard deviation and estimated LOD.**

Determination of the LOD is performed in the following steps:

- Estimating the LOD; Table.4
- Establishing the LOD; Table.5
- Evaluating the compliance ratio R.Table.6

Dye	*LOD (ppm)*
Amaranth	0.055
Azorubine	0.074
Brilliant Blue FCF	0.028
Patent Blue	0.031
Erythrosine	0.057
Indigo Carmine	0.034
Quinoline Yellow	0.036
Sunset Yellow	0.169
Allura Red AC	0.082
Ponceau Red	0.346
Tartrazine	0.112

Table 5: Limit of detection.

Dye	*Ratio of LOD*
Amaranth	5.985
Azorubine	4.442
Brilliant Blue FCF	9.137
Patent Blue	7.249
Erythrosine	4.130
Indigo Carmine	4.271
Quinoline Yellow	4.258
Sunset Yellow	4.512
Allura Red AC	4.791
Ponceau Red	4.515
Tartrazine	5.561

Table 6: The compliance ratio of the detection limit.

Calculating the ratio of compliance allows us to determine the validity of an approach to the establishment of a detection limit. was obtained as the calculation results for the ratio R which is used to establish a detection limit between 4 and 10 which means that the concentrations used were deduced that are adequate limits statistically obtained are good, and it is practically very low which makes operational same technique for the identification of dyes in united trace a sample.

Limit of quantification of method (LQM)

The limit of quantification of a method is the minimum concentration that can be quantified by using an analytical method with a defined reliability. This is the concentration equivalent to 10 times the standard deviation obtained during the establishment of the LOD.Table.7.

The limits of quantification were obtained by applying the Canadian protocol are between 0.094 and 1.67 ppm, which is used to quantify samples with low concentrations of dye with an assurance of the reliability of the results. This technique quantifies the very small amount of dye helps to the detection of fraud in food.

Dye	*LOQ (ppm)*
Amaranth	0.183
Azorubine	0.248
Brilliant Blue FCF	0.094
Patent Blue	0.106
Erythrosine	0.193
Indigo Carmine	0.116
Quinoline Yellow	0.121
Sunset Yellow	0.564
Allura Red AC	0.273
Ponceau Red	1.155
tartrazine	0.375

Table 7: Limit of quantification.

Linearity

The linearity of an analytical method is its ability to induce test results which are directly proportional to the concentration of analytes in samples in a proportional range or using mathematical transformations defined given. The linearity is demonstrated directly on standard of synthetic dyes (for dilution of the stock solution of 1000 ppm standard). Fig.3,4 and 5.

The linearity is determined by a series from May to October 2 or more injections of standards whose concentrations extend over 80 to 120 percent of the range of expected concentrations. The response is directly proportional to the concentration of the analytes. A linear regression equation applied to the results with an interception not significantly different from . Table.8.

This study verifies that the linearity of the colored solutions are in a concentration range where the responses are linearly proportional to the concentrations injected, this range is between 2.5 and 50 ppm, however it has been extended to 0.2 ppm to patent blue, the patented blue and erythrosine and this is due to their large enough answers. They were prepared in 50 mL vials from stock solutions of 50 ppm produced beforehand.

Dye	*Equation*	*R2*
Amaranth	y = 39.143x + 0,1459	0.9998
Azorubine	y = 29.318x + 4,6931	0.9991
Brilliant Blue FCF	y = 160.34x + 11,355	1
Patent Blue	y = 191.28x - 12,704	0.9999
Erythrosine	y = 114.46x + 12,544	0.9996
Indigo Carmine	y = 23.119x - 9,2156	0.9973
Quinoline Yellow	y = 60.249x - 47,165	0.999
Sunset Yellow	y = 15.266x - 4,4843	0.9988
Allura Red AC	y = 50.329x - 29,69	0.998
Ponceau Red	y = 27.41x + 1,9343	0.9983
tartrazine	y = 42.036x + 8,3108	0.9999

Table 8: Equations and correlation coefficients from linear curves.

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The values of the correlation factors obtained from lines made from the experimental values found in the tests reflect linearity proportional to changes in concentration tested since they are between 0.9973 and 1.

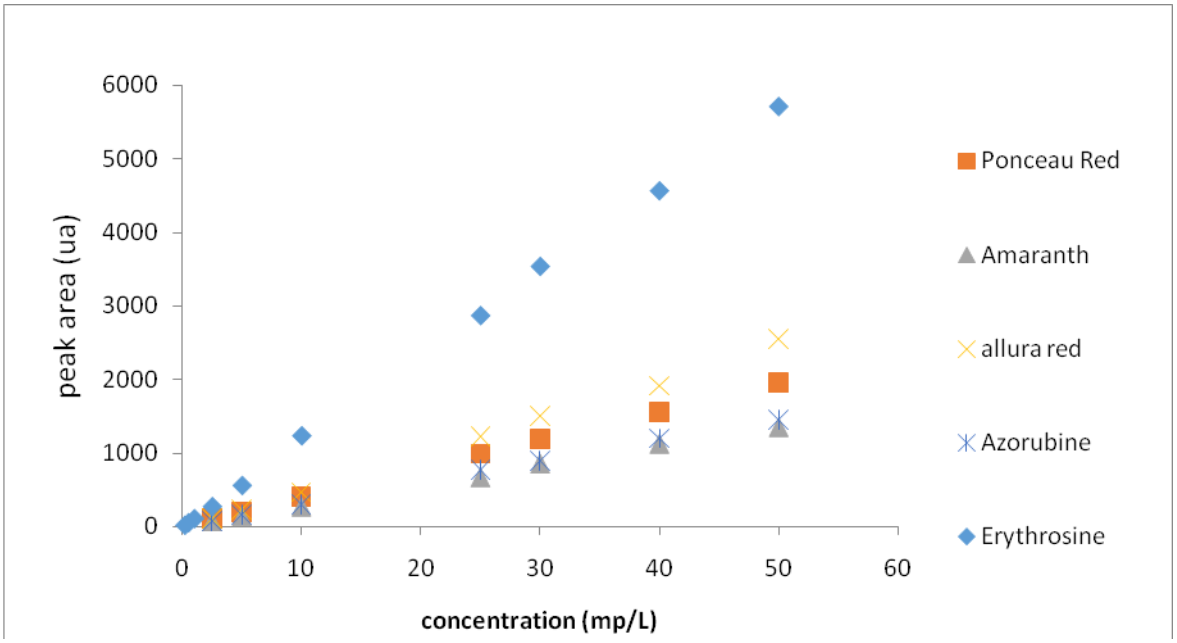

Figure 3: Linearity curves red dyes.

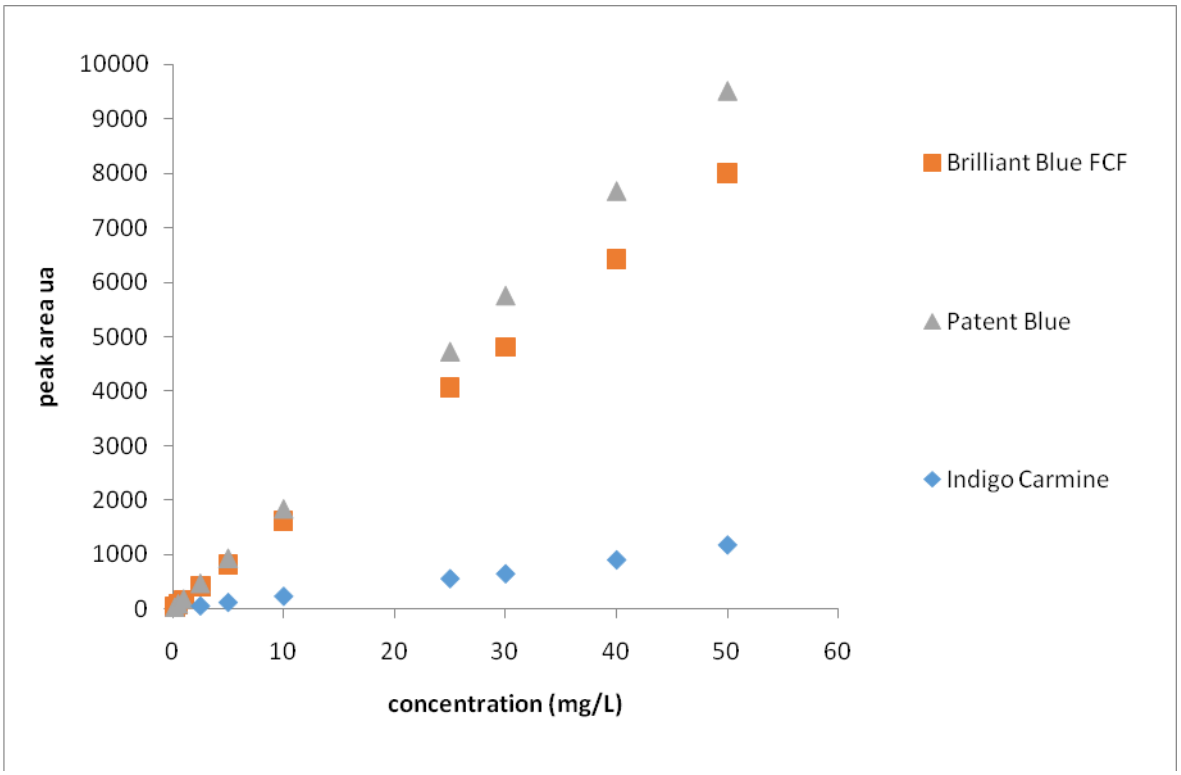

Figure 4: Linearity curves Blue dyes.

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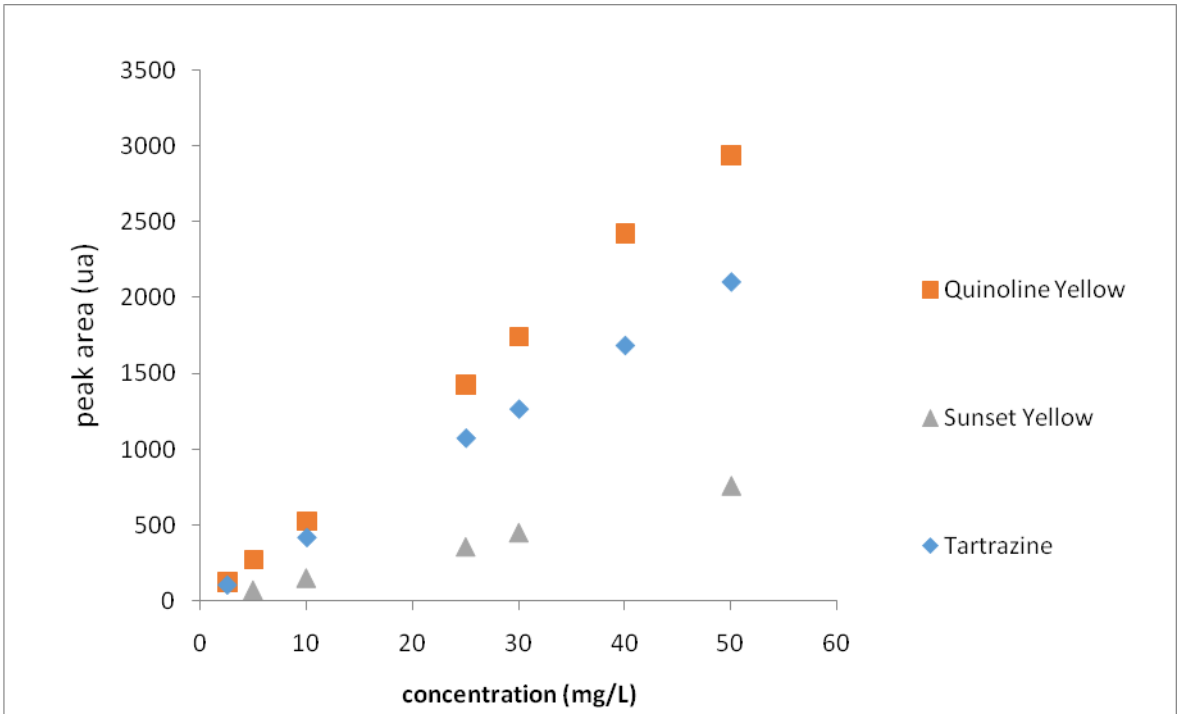

Figure 5: Linearity curves yellow dyes.

Fidelity

Loyalty to a given level is the closeness of agreement between the results obtained by applying the experimental procedure several times (n = 10 replica) under specified conditions. According to the conditions of execution of the test, this characteristic is expressed as replicability, repeatability for a method.

Replicability

Replicability at a given level is the closeness of agreement between successive individual results obtained on the same sample tested in the same laboratory under the following conditions: same analyst, same apparatus and same day.Table.9

Dye	*Replicability*	*coefficients of variation*
Amaranth	2.85	0.65%
Azorubine	5.82	0.55%
Brilliant Blue FCF	5.64	0.33%
Patent Blue	13.54	0.66%
Erythrosine	0.55	1.06%
Indigo Carmine	5.33	0.31%
Quinoline Yellow	14.15	0.75%
Sunset Yellow	2.56	0.32%
Allura Red AC	3.22	0.22%
Ponceau Red	7.87	0.93%
tartrazine	1.52	0.13%

Table 9: Replicability of eleven dyes and their coefficients of variation.

Repeatability

Repeatability at a given level is the closeness of agreement between individual results obtained on the same sample tested in the same laboratory and at least one of the following is different: analyst the device during the day.Table.10.

In general, the coefficients of variation should be less than 5%, and should rarely exceed 10% over the entire range. Coefficients of variation largely low rarely not exceeding 1% (one case Replicability Erythrosin with1, 06% and two other Erythrosin Repeatability 1.03% and 1.19% Ponceau Red) found that evidence that fidelity is very good.

Dye	*Repeatability*	*coefficients of variation*
Amaranth	1.81	0.41%
Azorubine	7.84	0.73%
Brilliant Blue FCF	7.14	0.41%
Patent Blue	6.43	0.31%
Erythrosine	13.88	1.03%
Indigo Carmine	6.57	0.37%
Quinoline Yellow	8.73	0.46%
Sunset Yellow	3.15	0.39%
Allura Red AC	6.35	0.43%
Ponceau Red	10.06	1.19%
Tartrazine	2.68	0.22%

Table 10: Repeatability of eleven dyes and their coefficients of variation

Accuracy

The accuracy at a given level is the closeness of agreement between certified by a recognized value and the mean result which would be obtained by applying the experimental procedure ten times (n = 10 replica). The accuracy is measured at a given level of concentration in the practice area quantifiable method. It is expressed as the relative error.Table.11.

The accuracy is the closeness of agreement between the average value obtained from a large series of test results and the conventional true value of the sample (the accepted reference value) whose experience has been demonstrated with values between 85.26 and 98.07 which indicates that this method is indeed just for a proper identification and quantification of 11 food colors simultaneously.

Dye	*relative error*	*ACCURACY %*
Amaranth	9.58	90.42
Azorubine	7.89	92.11
Brilliant Blue FCF	2.86	97.14
Patent Blue	14.74	85.26
Erythrosine	-4.95	95.05
Indigo Carmine	-2.31	97.69
Quinoline Yellow	1.38	98.62
Sunset Yellow	1.97	98.03
Allura Red AC	-3.53	96.47
Ponceau Red	2.11	97.89
Tartrazine	1.93	98.07

Table 11: The relative error and the accuracy percentage

Sensitivity

Sensitivity to a given concentration is the ratio of the magnitude of the measured variable with the corresponding value of the concentration of the element to be assayed.Table.12.

The sensitivity of a method that represents the slope of the calibration curve, so that the calibration curve is not a straight line, the sensitivity to a given concentration will be defined as the slope of the tangent to the curve at this concentration. Given that we found relatively high values for this parameter validation for all dyes, it is clear that it will be much easier to distinguish between several samples of neighboring concentration. The values obtained have allowed us to evaluate theoretically the values of instrumental detection limits since increased sensitivity allows for detection limits or lower quantification.

Dye	*slope. signal units / mg · l-1*
Amaranth	29.318
Azorubine	191.28
Brilliant Blue FCF	23.119
Patent Blue	60.249
Erythrosine	15.266
Indigo Carmine	50.329
Quinoline Yellow	27.41
Sunset Yellow	42.036
Allura Red AC	114.46
Ponceau Red	160.34
Tartrazine	39.143

Table 12: The sensitivity values obtained of the curve linearity.

Percentage of recovery

The percent recovery identifies, for a given sample or a given matrix type and a given level of concentration, the presence of potential interference during the analysis process. The recovery rate is the difference (in percentage) between the measured fortified sample and the measured concentration of the same unfortified sample, divided by the concentration of the added substance concentration. This report takes into account the chemical transformation that occurred, if any. A minimum of five tests required for evaluation of a method of analysis.Table.13.

The recovery rate is the difference (in percent) between the measured value of a sample which was prepared accurately vis-à-vis the concentration of dyes which have been put on this matrix concentration at which it was added to the saccarose since it does not constitute a source of interference and the fact it is suggested as a load-bearing joint in the circular of the Ministry of health and Ministry of Agriculture and Marine Fisheries and the measured concentration of the same sample. This report takes into account the chemical transformation that occurred, if any. And solved with rates that are greater than 84% can be deduced that in the recovery is good enough for a quantification method.

Dye	*PERCENTAGE OF RECOVERY*
Amaranth	95.04%
Azorubine	99.12%
Brilliant Blue FCF	86.67%
Patent Blue	90.10%
Erythrosine	91.76%
Indigo Carmine	94.37%
Quinoline Yellow	90.33%
Sunset Yellow	97.57%
Allura Red AC	96.98%
Ponceau Red	84.55%
tartrazine	92.91%

Table 13: Values of the recovery percentage.

CONCLUSIONS

HPLC-DAD method to identify and simultaneously quantify eleven synthetic food dyes frequently used in food products present in the Moroccan market was validated according to current guidelines of the Centre of Expertise in environmental analysis of Quebec and has been shown to be selective, linear, precise, accurate and reliable in the field of validity. The method has been demonstrated that the stability of indication of its ability to perfectly separate the dyes and detect fraud in colorful food matrices.

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