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Validation of LC-MS/MS for food colors in foodstuffs and household products

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ABSTRACT

The aim of this study was to develop a quadrupole liquid chromatography mass spectrometry (LC-MS/MS) method suitable for quantification of synthetic food colors, used as additives or occurring as adulterants in the food or cosmetics industries. Ten colors were validated in terms of limit of detection (LOD), limit of quantification (LOQ), precision, trueness and applicability. All tested parameters of the validation were within acceptable values, and the method was comparable to the existing high pressure liquid chromatography (HPLC UV-PDA) method.

1. Introduction

Food additives that are colorants, also known as food dyes, are used in the food industry as additives to homogenize color or to improve the visual quality of product. Sometimes they are also added to make color look more natural, since color stability fluctuates over time. Food colors can be synthetic, synthesized but equivalent to the natural color component, or naturally derived colorants. Their impact on human health is widely studied. Recent and older studies show that excessive exposure can cause cancer (*IARC,* 1975). The maximum levels of food colors in foods are defined in the European Union (*European Union Regulation*, 2023); this law is the update of the 2008 law on food additives. Certain colors have been allowed for use since December 2004 in meat products in Serbia, so in the following years, there was increased demand for development and use of adequate analytical methodology for determination of their presence and quantity in meat products (*Feng et al*, 2011). Different techniques are recommended for food analysis in terms of the presence and and amount of food colorants. One of the most convenient techniques is quadrupole liquid chromatography mass spectrometry (LC-MS/MS).

The aim of this study was to develop a LC-MS/MS method to quantify synthetic food colors, used as additives or occurring as adulterants in the food or cosmetics industries.

2. Materials and methods

Validation of the LC-MS/MS method for the determination of ten artificial colorants in food was performed on a triple quadrupole mass spectrometer, LCMS-8050 CL (Shimadzu Corporation, Japan).

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Tartrazine, Patent Blue, Ponceau 4R, Indigo Carmine, Sunset Yellow FCF, Allura Red AC, Azorubine, Green S, Brilliant Blue FCF, and Brilliant black, were obtained from Sigma-Aldrich (St. Louis, MO, USA). All of the stock solutions (1000 μg/mL) prepared were dissolved in HPLC grade water. Methanol and acetonitrile were purchased from Sigma-Aldrich. The ESI ionization was executed in either negative or positive mode. Under certain ESI conditions, all compounds showed higher sensitivity in negative than in positive mode; the most abundant ion was $[M–H]^-$ for all analytes. Each target analyte (10 μg/mL, in HPLC grade water) was tuned individually in order to obtain stable precursor and product ion abundance. Mixtures of colors from working solutions were added to blank sweetened gelatin mass to obtain concentrations of 10 mg/kg, 20 mg/kg, 50 mg/kg and 200 mg/kg of each dye. These samples were prepared according our standard operating procedure (SOP), and were examined along with certified reference materials (CRMs), and foods/household products with declared dyes purchased from the retail market. Samples were extracted before analysis according to the SOP: minced, blended and/or homogenized, then dissolved in an ultrasound bath with a mixture of ethanol-ammonia-water 7.5-2.0-0.5/v-v-v. Extracts of the samples were filtered through membrane filters with a pore size of 0.45 μm into the vials for the spectrophotometer's autosampler.

3. Results

Limits of detection and quantification of the method were estimated following the IUPAC approach, which consisted of analyzing the blank sample to establish noise levels and then estimating limit of detection (LOD) and limit of quantification (LOQ) for signal/noise ratio, 3 and 10 respectively (*Feng et al*., 2011). The LOQs and LODs for the food dyes are listed in Table 1. Also, intermediate precision and trueness were measured using reference materials for artificial food dyes (FCFA CON-32QC and other products, Fapas®)

Linearity of results was determined for all standard colors in the range of 0 mg/kg to 200 mg/kg in both water and blank gelatin. The term linearity of signal in LC-MS is used for determination of the linear relationship between analyte signals and analyte concentrations in calibration samples and the linear relationship between analyte signals and analyte concentrations in samples due to the matrix effect (*Chia-Fen et al*., 2015). For all samples, linearity was between 0.97 and 1.00.

Within-laboratory reproducibility of the results was determined by analyzing the samples listed in Table 2. Samples used for analysis were fruit- and vegetable-based products, candy and cake products, mouth rinse (a personal hygiene liquid) and dishwashing detergent. Six replicates of each sample were prepared, and the results were analyzed with

Table 1. LOD and LOQ values, intermediate precision and trueness for the LC-MS/MS method for determining synthetic food dye content, * Fapas® artificial food dye in gelatin-based sweets

Name of additive	LOD (mg/kg)	LOQ (mg/kg)	Intermediate Precision. STDEV	Trueness (%)
Additive E 102 (Tartrazine)*	0.46	0.52	7.32	96.7
Additive E 131 (Patent Blue)	0.52	0.53		
Additive E 133 (Brilliant blue)*	0.38	0.45	8.64	87.6
Additive E 142 (Green S)*	1.43	1.52	8.45	85.32
Additive E 151 (Brilliant Black)	0.86	0.87		
Additive E110 (Sunset Yellow FCF)*	0.59	1.25	10.22	94.4
Additive E122 (Carmoisine. Azorubine)	0.42	0.94		
Additive E124 (Ponceau 4R)	0.68	1.20		
Additive E129 (Allura red AC)*	0.70	1.42	11.32	84.45
Additive E132 (Indigo carmine)	0.75	1.39		

Table 2. Validation parameters for LC-MS/MS to quantify synthetic food dyes, showing repeatability, reproducibility, and standard deviation

Sample	Analyte – synthetic food dye	Synthetic food dye content, measured by LC-MS/MS (mg/kg)	Within laboratory reproducibility	t_{crit}	|t|	STDEV (LC-MS/MS HPLC-UV PDA)
Surimi	E 120	5.67	0.36	2.57	0.71	9.10
Fruit dessert	E 102	47.55	1.26	2.57	0.36	8.38
Frozen peas	E 142	18.81	0.09	2.57	0.09	10.41
Fusilli	E 142	27.61	0.74	2.57	0.87	8.66
Butter biscuit	E 142	36.22	0.34	2.57	0.95	8.48
Lolly pop	E 102 E 124	40.71 58.74	0.17 0.48	2.57	1.41 0.38	6.47 9.87
Fruit yogurt	E 102	32.24	0.56	2.57	0.27	13.87
Tomato sauce	E 129	19.07	0.13	2.57	1.37	9.33
Baby food	E 102	20.08	1.08	2.57	0.84	7.17
Orange syrup	E 110	45.05	0.54	2.57	0.52	11.25
Mouth rinse	E 131 E151	32.69 24.29	0.29 0.34	2.57	1.52 1.29	8.03 11.45
Dishwashing detergent	E 102 E 133	37.28 7.51	0.27 0.91	2.57	0.38 2.07	6.37 9.74

T-test to compare the difference between replicates. All samples were commercially available on the local market and labeled by manufacturer regarding color content. Reproducibility for all products examined was far lower than the critical T-value, showing good reproducibility of the results by the LCMS method (Table 2).

Dwell time for all standards was 10 ms. Precursor ions, product ions and collision energy are listed in Table 3.

Table 3. LC-MS/MS chromatographic results and conditions

Food dye	Precursor m/z	Product ion m/z	Collision energy
Patent blue E131	559.2	435.3479.5	62.045.0
Brilliant blue E133	747.4	171.0561.0	79.061.0
Green S E142	553.3	416.0496.0	49.038.0
Azorubine E122	227.9	169.9220.9	22.018.0
Allura red E129	225.1	207.0136.0	22.034.0
Sunset yellow E110	407.1	207.1327.1	45.030.0
Ponceau 4R E124	267.9	301.9205.9	15.017.0
Indigo carmine E132	226.0	198.0105.0	27.053.0
Tartrazine	210.9	197.9170.9	15.015.0
Erythrosine E127	834.8	663.0537.0	52.054.0

Those values were initially transferred from existing methods but during the process of validation were changed, either undergoing a complete change or just slight shift of values (*Ntrallou et al*., 2020). Most of transitions that were dominant were chosen for further chromatographic conditions and used in validation process.

3.1 Comparison of LC-MS/MS to LC-UV-PDA validation results

LC-MS methods have the advantage of higher sensitivity, higher selectivity and higher throughput compared with LC-UV methods (*Kim et al*., 2018). Nevertheless, the previously developed existing high pressure liquid chromatography (HPLC UV-PDA) method was comparable to the new LC-MS/MS method, since standard deviations for samples analyzed with the two different methods were lower or about 10%.

4. Conclusion

The LC-MS/MS method developed and validated for determination of food colors is convenient and suitable for routine analysis of dyes in different food products and other household products that might affect human health. The method is comparable to our existing HPLC UV-PDA method, according to results obtained by analyzing different retail products from the market. Trueness and reproducibility are acceptable and reproducible compared to assigned values from reference materials.

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