Green Analytical Chemistry 3 (2022) 100024

Contents lists available at ScienceDirect

Green Analytical Chemistry

journal homepage: www.elsevier.com/locate/greeac

Evaluation of the sample treatment influence in green and sustainable assessment of liquid chromatography methods by the HEXAGON tool: Sulfonate-based dyes determination in meat samples

A. Ballester-Caudet, R. Navarro-Utiel, I. Campos-Hernández, P. Campíns-Falcó*

MINTOTA Research Group, Departament de Química Analítica, Facultat de Química, Universitat de València, 46100, Burjassot, Valencia, Spain

ARTICLE INFO

Keywords:
Hexagon pictogram
Greenness
Sustainability
Miniaturization
in-tube SPME
Food
Dyes

ABSTRACT

Green and Sustainable assessment of sample treatment has been carried out when using liquid chromatographic methods in food analysis. Particularly, published sulfonate-based dyes determination by conventional liquid chromatography with diode array (HPLC-DAD) or mass detectors (HPLC-MS/MS) was selected as starting point. Greenness-sustainability have been quantitatively evaluated by means of the HEXAGON tool, in which sample treatment and method characteristics noticeably play a key role in defining the figures of merit of the analytical procedure. According to the evaluation results that are represented in a hexagon pictogram with an overall score from 0 to 4 (the lower the score, the greater contribution), a new analytical method is proposed by means of both minimizing and miniaturizing sample treatment step. In-tube solid-phase microextraction online coupled to capillary LC (IT-SPME-CapLC-DAD) provided a more green and sustainable alternative to current food dyes analysis without losing sensitivity (LODs 0.5-1 μg/g sample). The proposed method was tested on chicken meat samples (2 g), using ethanol-ammonia-water mixture (80:1:19, v/v/v; 5 mL) as extraction solution. The arithmetic means of the HEXAGON scores (s_{av}) were: 1.71, 2.57 and 2.71 for IT-SPME-CapLC-DAD, HPLC-DAD and HPLC-MS/MS, respectively. The proposed method was closer than the others tested to a hypothetical LC procedure without sample treatment, which provided a s_{av} value of 1.

1. Introduction

The concept "sustainable" is related with the idea of reconciling economic development and the conservation of natural ecosystems; it was first appeared in 70's [1]. The term sustainability reached in 1987. Its current concept first appeared in the Brundland report (Our Common Future Report) in 1987 by the World Commission on Environment and Development [2]. This report warned about the negative environmental consequences of economic development and globalization, trying to offer solutions to the problems derived from population growth and industrialization. From then until today, this term sounds more and more frequently and is linked to many areas.

Green chemistry was born in the Environmental Protection Agency (EPA) in USA in the early 1990s as an approach and a conceptual tool for environmental protection to pollution caused by chemistry industry, and was expressed by known 12 principles established by Anastas and Warner in 1998 [3]. Green chemistry is the design, development and implementation of chemical processes or products to reduce or eliminate the use and generation of dangerous and toxic substances [4]. Sustainable chemistry also considers the effects of processing, materials, energy, social and economic impacts [5]. In general, sustainable chemistry should use resources, including energy, at a rate at which they can be replaced naturally, and the generation of waste cannot be faster than the rate of their remediation. There is an intersection zone between both mentioned subjects, greenness and sustainability, both included into the wider concept of suitable chemistry [5].

Various efforts taken by the scientific community have relied on building an analytical eco-scale to evaluate the greenness of analytical methods [6–8]. Although successful algorithms and pictograms have been achieved for evaluating the performance of routine analyses, a global evaluation that accounts not only for environmental criteria but also productivity of the method and its economic efficiency, ease of use and safety is highly desirable. All in all, the HEXAGON tool [9,10] jointly considers Green chemistry principles in cooperation with environmental impact and economic cost aspects to quantitatively assess sustainability of analytical methods. Objective criteria are evaluated through the definition of penalty points divided into five different blocks, namely, figures of merit, toxicity and safety, residues, carbon footprint and economic cost. For each block, the overall qualification is scaled from 0 to 4 and it is depicted on a regular hexagonal pictogram (see Fig. 1) that

Abbreviation: IT-SPME, In-tube solid phase microextraction.

* Corresponding author.
E-mail address: pilar.campins@uv.es (P. Campíns-Falcó).

https://doi.org/10.1016/j.greeac.2022.100024
Received 19 May 2022; Received in revised form 20 July 2022; Accepted 24 July 2022

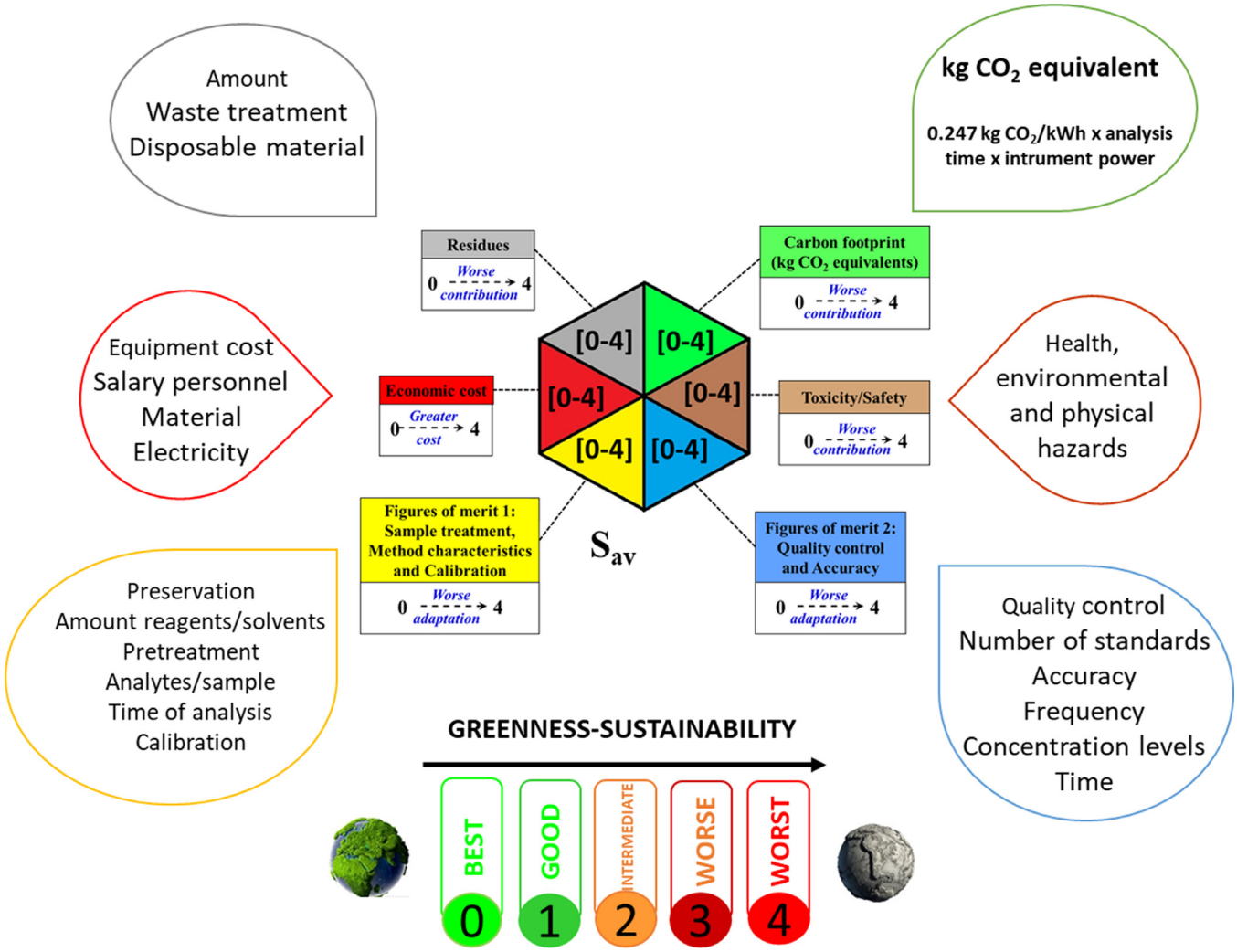

Fig. 1. Regular hexagon pictogram divided into six blocks for method evaluation. S_{av} accounts for the arithmetic mean of the 0-4 score for each block (toxicity and safety are computed separately).

allows a user friendly comparison of analytical procedures. Eventually, the arithmetic mean (Sav) of the 0-4 scale is computed in order to compare analytical methods from a single data. HEXAGON is in line with Green and Sustainable chemistry philosophy, balancing also the figures of merit needed for solving a given problem. The lower the score, the better the adaptation of the analytical procedure to greenness and sustainability aspects that lead to a reliable analytical result.

HEXAGON was compared in [10] with other two tools: multi-criteria decision analysis (MCDA) algorithms that result in an indication of the best procedure to solve a given specific analytical problem and an RGB model, named so because of the analogy to the model commonly used in representation and coding of colors in electronic devices. Besides, from this last tool, an approach named White Analytical Chemistry (WAC) was introduced to reconcile the principles of Green Analytical Chemistry and functionality [11]. Recently, review articles and book chapters have been published covering greenness of analytical methods [12–14].

In this paper we studied how sample treatment can impact greenness and sustainability of liquid chromatography methods from the HEXAGON tool and, in the context of several procedures for controlling color additives in food. Food chemistry widely uses synthetic dyes in beverages, juices, meat products and sweets to preserve or alter natural colour of food and, hence, improve its appearance [15–17]. Within the framework of meat and feed production industries, food additives play a key role due to their low price and high stability; however, some of these substances and their metabolites pose human health risk when consumed in excessive amounts [18] giving rise to several adverse effects such as allergies, asthmatic reactions and carcinogenicity, among others, that impose strict control analysis [19]. In this regard, European legislation establishes the so-called Acceptable Daily Intake (ADI) values with the aim of providing a guideline for determining the healthy maximum amount of food dye that can be daily ingested, expressed on a bodyweight basis as mg of colorant per kilogram of body weight (bw) per day. Regarding the most employed sulfonate-based synthetic dyes, current established ADIs regulated by the European food safety authority (EFSA) [20] are listed in Table 1 together with Erythrosine.

In the literature, it was found that synthetic dyes determination has been usually performed by conventional separation techniques such as high performance liquid chromatography with diode array (HPLC-DAD) or mass detectors (HPLC-MS/MS) when analyzing meat samples [21]. These conventional chromatographic methods require a preliminary extractive procedure prior to analytical separation in order to reduce matrix complexity and/or analyte preconcentration, especially in meat products. The extraction procedure normally involves the use of organic solvents followed by a pre-concentration step throughout solid phase extraction (SPE) or evaporation and redissolution in a smaller volume due to the trace concentration levels of dyes in meat samples.

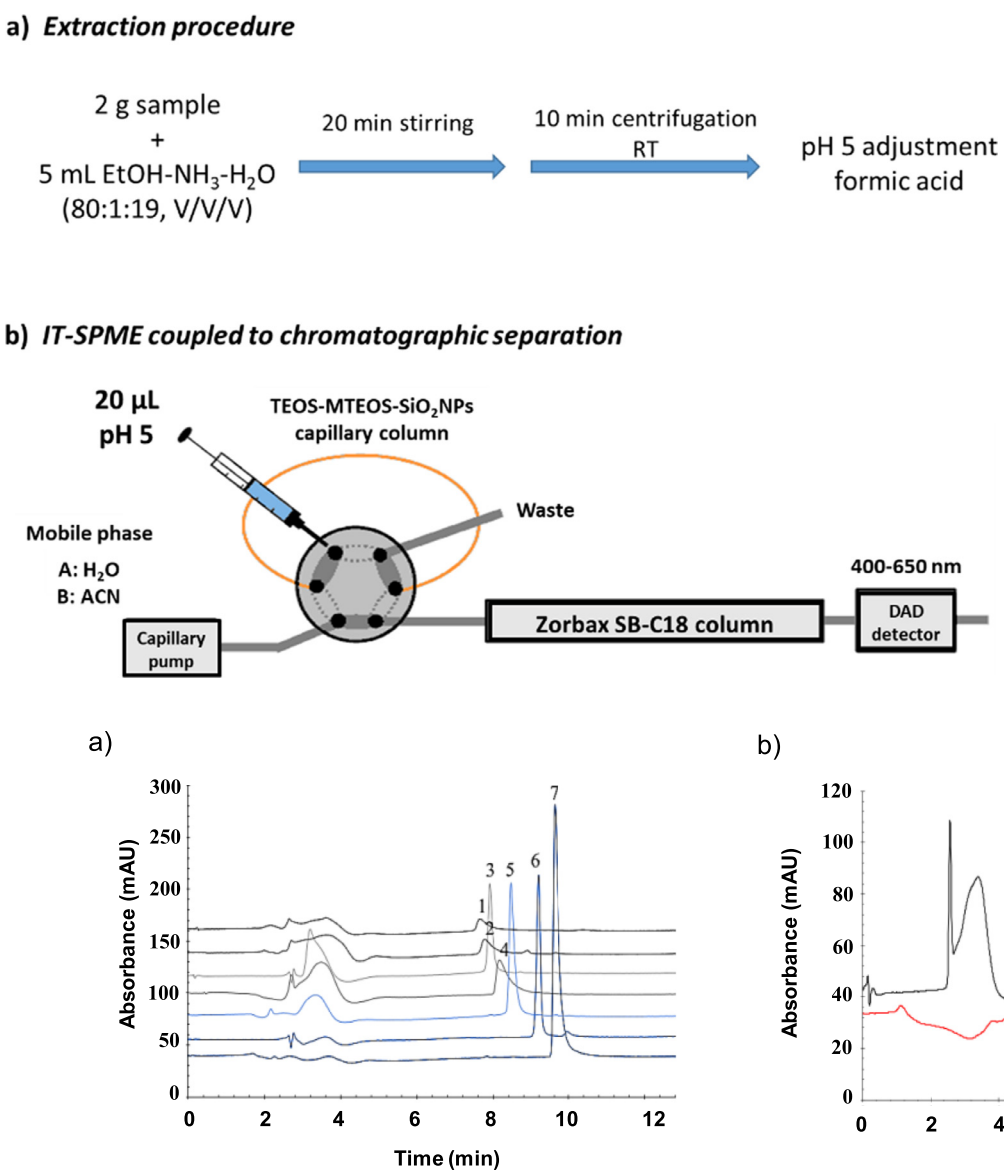

Fig. 2. Schematic diagram of the in-tube SPME-CapLC-DAD system using a 6-port valve.

Fig. 3. Chromatograms obtained for: **a)** Sunset yellow (1), New coccine (2), Acid red 1 (3), Allura red (4), Ponceau xylidine (5), Orange II (6) and Erythrosine (7) at a concentration of 2.5 ppm and pH 5. **b)** Multicomponent mixture of the analytes of 1 ppm with (red) and without (black) washing the capillary column loop from IT-SPME with 20 µL of ultrapure water. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Sulfonate-based food colorants ADIs values declared by the EFSA.

Food colorant	ADI (mg/kg bw[a]/d)
Sunset yellow (E110)	0-2.5
New coccine (E124)	0.7
Acid red 1 (E128)	0.1
Allura red (E129)	7
Erythrosine (E127)	0-0.1

[a] bw (body weight).

Therefore, sample treatment turns out to be a crucial step for a successful analyte determination.

Data related to the food dyes quantification in foodstuffs are lacking. Regarding the sector 'meat and meat products', this gap is also due to the fact that the attention of official controls is more focused on the quantification of other food additives in respect to food dyes [22]. However, the use/abuse of food dyes in meats has to be constantly monitored, due to several food safety concerns. Iammarino et al. [22] made a survey of dye use in fresh meat preparations and meat products by HPLC-DAD.

In this work, the HEXAGON tool has been applied to assess greenness and sustainability of synthetic dyes determination by conventional HPLC-DAD or HPLC-MS/MS methodologies previously published [21]. These LC HEXAGONs were compared with that corresponding to a hypothetical LC method without sample treatment. Besides, a new analytical method has been developed based on in-tube solid phase microextraction (IT-SPME) coupled on line to capillary LC-DAD [23–26], with the aim of improving aspects in line with the parameters defined within the framework of the HEXAGON tool. Sample preparation is still a critical step in many analyses. It is estimated that about 65% of the total of samples analyzed require three or more operations in order to obtain a suitable instrumental response. In addition, sample preparation often involves the consumption of large amounts of solvents, reagents, materials, and energy. However, it is encouraged to use solvent-less extraction techniques and/or the introduction of less toxic solvents. Therefore, it is a goal to decrease the time required for this analysis step and to achieve it in a green and sustainable manner. In this sense, IT-SPME

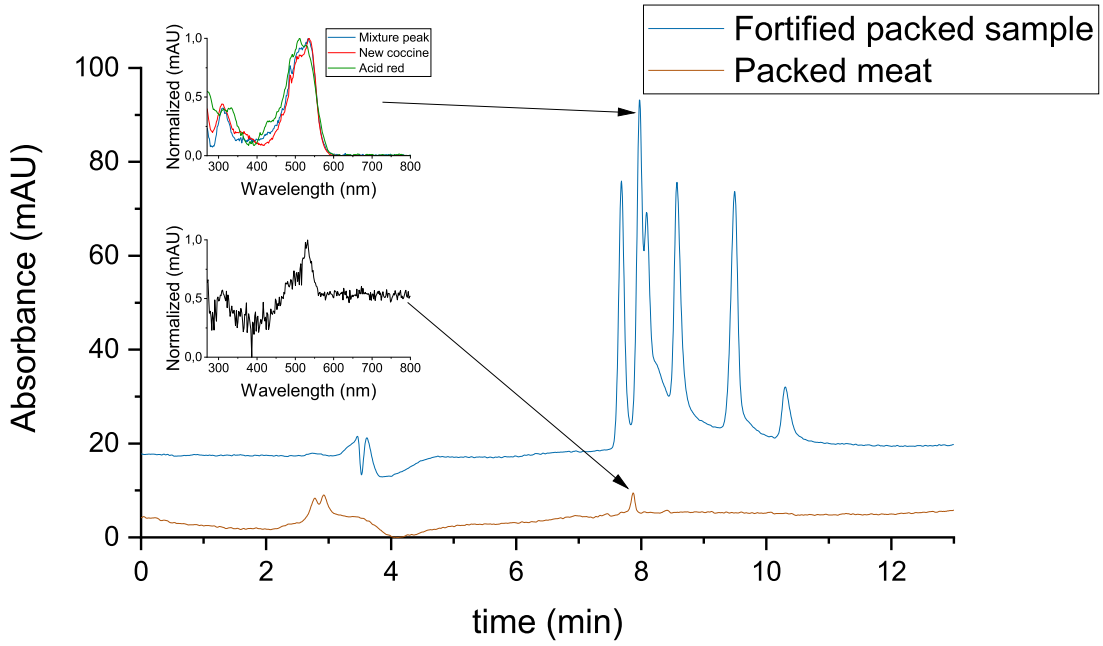

Fig. 4. Chromatograms obtained for the chicken sample (red) and spiked chicken sample (black) at 2.5 ppm of a seven multicomponent dye solution. The 8-minutes retention peak provided an absorbance spectrum depicted in the inset. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

is an attractive technique. The potential of IT-SPME arises mainly from its versability for on-line coupling to LC. Initially, IT-SPME was coupled with conventional LC. More recently, research aimed at developing the coupling of IT-SPME with ultrahigh performance LC, capillary LC, and Nano-LC is worthy of mention. MINTOTA research group described by the first time coupled in-tube SPME with new chromatographic modalities and new capillary coatings [25].

Interestingly, an optimization of sample treatment step and some method characteristics have been proved to offer a more green and sustainable alternative procedure for determination of dyes in food analysis. The applicability of the proposed method to real sample analysis has been verified in meat samples.

2. Materials and methods

2.1. Reagents and samples

Sunset Yellow, New Coccine, Acid Red 1, Allura Red, Xylidine Ponceau, Orange II and Erythrosine synthetic dyes were purchased from Sigma-Aldrich (Merck, Missouri, USA). Methanol (MeOH), ethanol (EtOH) and acetonitrile (ACN) organic solvents were obtained from VWR Chemicals (Pennsylvania, USA). Formic acid and 25% ammonia were provided from Merck (New Jersey, USA) and Scharlau (Barcelona, Spain), respectively. Ultrapure water was obtained from Thermo Scientific™ Banstead™ Nanopure water purification equipment from Thermo Fisher Scientific (Massachusetts, USA) with a resistivity of 18.2 MΩ cm and a total carbon number (TOC) less than 2 ppb.

Chicken meat samples were purchased from local market in Valencia (Spain). Standard and sample solutions were prepared with ultrapure water. Nylon syringe filters (0.22 μm pore size and 13 mm diameter) provided by Epica S.L. (Valencia, Spain) and Labbox (Barcelona, Spain), respectively, were used for the sample extracts.

2.2. Instrumentation and chromatographic conditions

Pretreatment and extraction procedures were performed using EBA20 model centrifuge from Hettich Zentrifugen (Tuttlingen, Germany), a digital magnetic stirrer model MS7-H550-Pro from Onilab (California, USA) and a desktop pH-meter model PH50+ from XS instruments (Modena, Italy).

Synthetic dyes stability in aqueous/organic solvent solution was studied by registering UV-Vis spectra through a UV-Vis Cary 60 spectrophotometer from Agilent Technologies (California, USA).

HPLC-DAD from [21]. Chromatographic separation of 20 µL dyes mixture was carried out using Waters Atlantis T3 column (2.1 mm x 150 mm, 3 µm). Mobile phase was composed of 20 mM ammonium acetate solution (A) and acetonitrile (B) with a constant flow of 0.2 mL/min and monitoring at 484, 526 and 550 nm wavelengths. Gradient elution mode was employed giving rise to a total analysis time of 16 minutes.

HPLC-MS/MS from [21]. Water Atlantis dC18 column (2.1 mm x 150 mm. 5 µm) was used for the chromatographic separation when injecting 10 µL of sample solution with a constant flow of 0.3 mL/min. Optimized conditions for electrospray ionization were: drying gas temperature 350 °C, gas flow 10 L/min, sheath gas flow 12 L/min and capillary voltage 3500 V. Mobile phase and gradient elution mode were similar to the ones employed for HPLC-DAD.

Cap-LC-DAD. Chromatographic separations of dyes mixtures were performed by using a 1100 series capillary liquid chromatograph (Agilent Technologies, Waldbronn, Germany): G1276A capillary pump, G1379A micro vacuum degasser. An Agilent Zorbax SB-C18 column (150 mm x 0.5 mm. 5 µm) and a diode array detector (DAD) 1260 infinity series G1315D were employed for the analysis. 20 µL of samples were processed using a six-port manual injection valve. The mobile phase used was ultrapure water (A) and acetonitrile (B) in gradient mode with a constant flow of 10 µL/min and monitoring at three specific wavelengths: 484, 526 and 550 nm. Specifically, spectra were recorded between 400 and 650 nm for qualitative purposes. The tested gradients are given in Table 2.

3. Results and discussion

3.1. Sample treatment procedure

The proposed analytical method is based on a greener extraction procedure than that used in conventional HPLC-DAD and HPLC-MS/MS

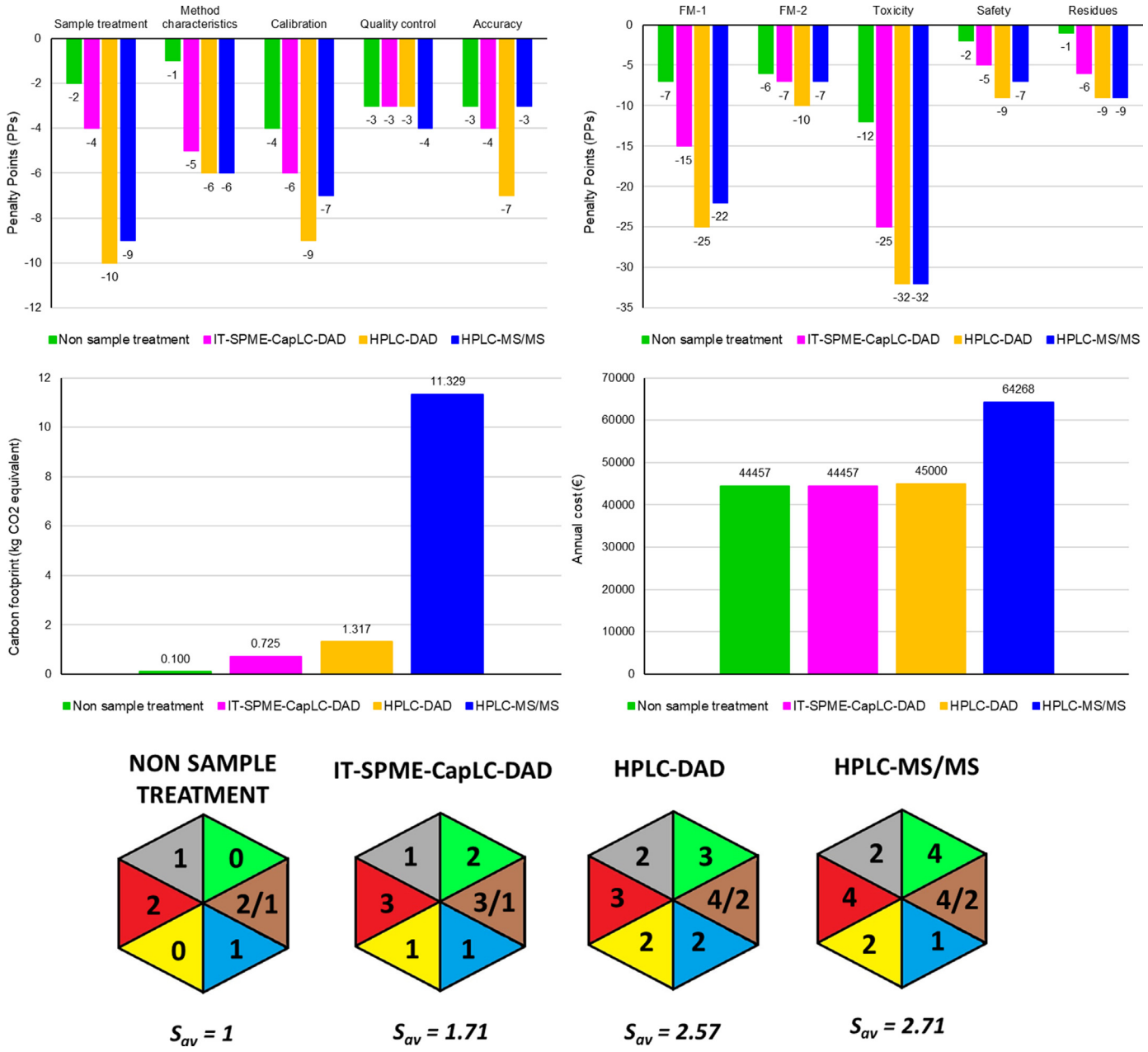

Fig. 5. Penalty points assessment for figures of merit 1 and 2, toxicity, safety and residues (a,b), carbon footprint expressed as kg CO_2 equivalent (c) and annual economic cost (expressed as euros) associated with the studied analytical methods and an hypothetical LC method without sample treatment. HEXAGON pictograms and s_{av} for the several methods tested.

Table 2
Gradients studied during the optimization of the IT-SPME-CapLC-DAD.

Gradient 1		Gradient 2		Gradient 3		Gradient 4	
ACN (%)	Time (min)	ACN (%)	Time (min)	ACN (%)	Time (min)	ACN (%)	Time (min)
5	0	5	0	5	0	5	0
40	6	40	2	25	4	40	4
90	9	40	4	60	5	90	9
90	11	90	9	95	10	90	11
5	13	90	11	95	12	5	13
		5	13	5	14		

Table 3
Degradation factor obtained in water and methanol solvents.

Analytes	λ_{max} (nm)	Absorbance MeOH	Absorbance H_2O	Degradation (%)
Sunset yellow	480	0.495	0.526	6
Acid red 1	505	0.412	0.385	7
Ponceau xylidine	500	0.467	0.451	3
New coccine	510	0.403	0.347	14
Orange II	485	0.623	0.618	1
Allura red	505	0.955	0.508	47
Erythrosine	530	0.890	0.043	95

methods [18], followed by a preconcentration step within the in-tube SPME assembly coupled to capillary LC. As shown in Fig. 2a, 2g of chicken sample are mixed with 5 mL of ethanol-ammonia-water (80:1:19, v/v/v) extracting mixture and the resulting mixture is kept under stirring for 20 min. When comparing to the conventional methods, it is worth mentioning the reduction of both the volume of ternary ethanol-ammonia-water extraction medium from 10 mL to 5 mL and a decrease of the stirring time of 10 min. In line with the conventional methods, the resulting mixture has to be centrifuged at 12.000 rpm for 10 min at 4 ˚C to eliminate precipitated proteins. In this work, we propose a simpler sample treatment by centrifuging the resulting extractive mixture at 6000 rpm only for 10 min at room temperature. At this point, the conventional preconcentration step implies drying 2.5 mL of supernatant at 50 ˚C in a water bath under nitrogen stream followed by residues redissolution with 0.5 mL of extracting mixture and pH 5 adjustment with formic acid for HPLC-DAD. Our proposed method minimizes the preconcentration step complexity by directly adding formic acid to adjust pH 5 and avoiding the aforementioned thermic sample treatment, which is substituted by the processing of the solution into the in-tube microextraction (IT-SPME) assembly. Previous injection to the capillary system, the solution is filtered using a 0.22 μm pore size membrane filter and 20 μL of solution are processed through the loop-shaped IT-SPME capillary column of tetramethylorthosilicate (TEOS), trimethoxymethylsilane (MTEOS) and SiO_2 nanoparticles (NPs) with a thickness of 350 nm (0.32 mm internal diameter) with a length of 20 cm via the 6-port valve [23,26], being its volume 16 μL. IT- SPME procedure on-line coupled to capillary LC was used for the chromatographic separation, as depicted in Fig. 2b. The influence of sample treatment in the greenness evaluation of the methods is assessed when applying the HEXAGON tool, as detailed in Section 3.4.

3.2. Optimization of chromatographic conditions

First, stability of the sulfonate-based dyes in aqueous medium was studied by UV-Vis spectrometry and compared to the one observed in commonly used organic solvents such as methanol [21,27]. UV-Vis spectra for 10 ppm reference solution of each synthetic dye were registered during a period of time of 45 days. Percentages of degradation and maximum absorbance obtained for each dye are listed in Table 3. It was found that most of the analytes present similar stabilities in both aqueous and methanol solvents except for Allura red and Erythrosine dyes, which showed a degradation factor higher than 45%. This implies these two dyes are recommended to be prepared and stored in methanol whereas water is the preferred solvent for the rest dyes.

Regarding the analytical separation, optimized chromatographic conditions were investigated when injecting 20 μL of a multicomponent solution at 2.5 ppm concentration for each analyte. The analytical identification was made by spectra comparison between the spectral window ranging from 400 to 650 nm. As listed in Table 2, several gradients were tested, all of them starting with a mobile phase composition of 5% acetonitrile. Gradient 4 provided the best separation and it was selected to perform the following experiments. The chromatograms obtained for single-component solution of each analyte are shown in Fig. 3a. It can be seen that suitable analyte identification and separation were obtained.

Table 4
Analytical parameters obtained for the different food dyes.

Analyte	Calibration curve	R^2	LOD (ppm)	RSD (%)
Sunset yellow	y = 55.273x −2.451	0.99	0.2	17
New coccine	y = 44.587x + 3.095	0.98	0.3	7
Acid red 1	y = 51.571x + 1.714	0.99	0.4	7
Allura red	y = 98.39x − 23.474	0.95	0.3	13
Ponceau xylidine	y = 109.06x + 7.755	0.99	0.3	19
Orange II	y = 102.74x + 1.417	0.99	0.3	23
Erythrosine	y = 154.99x + 22.535	0.99	0.3	17

In addition to analyte peaks, chromatograms show non-identified peaks at an estimated retention time of 3.5 min, as shown in Fig. 3a. In order to eliminate non-desirable peaks and improve analytical column life, washing the capillary column of the IT-SPME assembly with 20 μL of ultrapure water was tested. As shown in Fig. 3b it was an effective solution if necessary, although it implied sensitivity decrease.

As can be seen in the chromatogram from Fig. 3b, the number of peaks obtained for the seven multicomponent solution provided only six peaks, which suggests there is an overlap between the signals of a dye pair. For the sake of identification, different mixture pairs of dyes with similar retention times were injected. It was concluded that the overlapping signals were associated with peaks 2 and 3 from Fig. 3a corresponding to New coccine and Acid red 1 dyes. On the other hand, it should be noted that Sunset yellow and Orange II signals (peaks 1 and 6 from Fig. 3a) suffer a significant absorbance decrease when 526 and 550 nm depending on the wavelength used for the identification. In both cases 526 nm provides higher absorption values than 550 nm. This facilitates the analytical assignment and verification of peaks when considering the UV-Vis spectra of the analytes.

Some instrumental figures of merit obtained for the different analytes studied are indicated in Table 4. As can be seen, the calibration curves of analyzed dyes showed good linearity with satisfactory precision (RSD). Additionally, adequate limits of detection confer competing sensitivity of the proposed IT-SPME-CapLC-DAD method in comparison to conventional methods [21].

3.3. Synthetic dyes determination in meat samples

2 g of chicken meat were subjected to the extraction procedure described in Section 3.1. The chromatogram obtained can be seen in Fig 4. A signal close to the quantification limit was obtained at a retention time of 8 minutes. Then, the sample was fortified with 2.5 ppm of a 7-dye multicomponent standard mixture prepared a week before using and the analytical procedure was performed. Note that Erythrosine was degraded in the mixture (see also Fig 3b); as it is shown in Table 2, its stability in water is low. It was concluded that the peak observed in the sample can be assigned to New coccine considering its retention time and in accordance with its obtained spectrum (see insert of Fig 4). This dye, also called Ponceau 4R (E124) is one of the most employed red dyes in meat products [22]. The amount found is below established ADI value (see Table 1).

Table 5
Experimental procedures developed for synthetic dyes determination.

Sample preparation	Method characteristics	Calibration	Quality control and accuracy	Analysis
Macroscopic size 10 mL EtOH-NH_3-H_2O 30 min stirring 50 °C water bath N_2 stream pH 5 formic acid	Off-line Destructive 16 min analysis	Daily calibration ≤ 30 min required time 5-7 number of standards	≤ 30 min required time Poor accuracy magnitude Limited selectivity 30 min −1 h required time Good accuracy magnitude Optimal selectivity	HPLC-DAD HPLC-MS/MS
Macroscopic size 5 mL EtOH-NH_3-H_2O 20 min stirring Room temperature pH 5 formic acid	On-line Destructive 9 min analysis	Daily calibration ≤ 30 min required time 5-7 number of standards	≤ 30 min required time Adequate accuracy magnitude Adequate selectivity	IT-SPME-CapLC-DAD

3.4. HEXAGON tool for evaluation of analytical methods

The analytical parameters related to the sample treatment, method characteristics and quality control were assessed quantitatively and summarized in Table 5. According to the HEXAGON tool, the penalty points obtained when evaluating figures of merit 1 and 2 (FM-1 and FM-2) are shown in Fig 5. Within the figures of merit 1 block, the variables related to calibration: frequency, calibration time, number of standards, linear concentration range, limit of detection (LOD) and precision, the HPLC-MS/MS method could be considered the best choice for the determination of food colorants bearing in mind LODs and optimal selectivity. However, the proposed IT-SPME-CapLC-DAD method is more sustainable option that conventional methods since it requires fewer amount of the extracting mixture and replaces the mobile phase A used in conventional methods by water, which decreases the penalty points associated with the toxicity. Moreover, the time of analysis has been shortened under the optimized gradient elution mode.

Regarding the cost-effectiveness, an estimation of the annual cost was carried out for each method, taking into account the reagents, material, electricity consumption and the cost of personnel required to carry out the analytical determination for one year. A total amount of 50 weekly samples was assumed and the salary assigned to qualified personnel was fixed to 15 euros per hour as constants for the three methods studied. As can be seen in Fig. 5, the IT-SPME-CapLC-DAD procedure presents a very low carbon footprint due to the minimization of the sample treatment step and the decrease in the time of analysis. Also, the amount of waste generated is noticeably lower than in the other cases. Despite these advantages, it should be mentioned that the annual cost associated appears to be similar to HPLC-DAD, mainly because of the equipment cost itself. Sample treatment can remarkably improve the greenness and sustainability of an analytical method as it can be seen in Fig 5 considering the hypothetical method without sample treatment. IT-SPME-CapLC_DAD by means of both minimizing sample treatment and miniaturizing this step achieved better results than that provided with conventional LC procedures for the studied topic.

Finally, the sum of the PPs and estimated carbon footprint and cost values are ranked in an overall quantification for each variable using a 0-4 scale and organized in a hexagon (see Fig. 1) as resulting pictogram [9]. The scale is related with excellent, good, suitable, weak and fail performance of the tested analytical method for the scores: 0, 1, 2, 3 and 4, respectively. The higher the score (that is, getting closer to 4), the following statements are accomplished: the worst the adaptation of the figures of merit for providing a reliable analytical result, the worst the contribution to health and safety, the worst the environmental impact, sustainability and cost-benefit relation. Overall hexagon pictogram scores obtained from the HEXAGON tool in addition to the arithmetic mean (Sav) computation [10] allow to easily compare the methods by simple visual inspection. In Fig. 5, the pictograms obtained for each of the methods studied are shown, as well as the corresponding arithmetic mean including besides a hypothetical LC method without sample treatment, which provided a s_{av} value of 1. In accordance with the scores obtained in the hexagon pictograms and s_{av} values shown in Fig. 5, it can be stated that the proposed IT-SPME-CapLC-DAD procedure is a potential sustainable and green alternative to conventional methods for determining sulfonate-based synthetic dyes in routine meat analysis. Particularly, residues, carbon footprint, toxicity/safety and figures of merit were improved. Sample treatment impacted as it can be seen in Fig 5 in carbon footprint, toxicity, FM-1 and economical cost.

4. Conclusions

In the present work, the HEXAGON evaluation tool has been applied to compare conventional analytical procedures and a novel method for the determination and quantification of food dyes in meat products. The proposed IT-SPME-CapLC-DAD analytical procedure has been applied for synthetic dyes analysis, decreasing the amount of waste generation, minimizing the amount of reagents employed with a lower environmental impact. This work demonstrates that minimizing sample treatment and miniaturizing the system can remarkably improve the greenness and sustainability of an analytical method. In order to propose new analytical methodologies, the HEXAGON tool provides successful guidelines to reach the best option.

CRediT author statement

P. Campíns-Falcó: conceptualización, supervision, methodology, investigation, resources, writing-review&editing. **A. Ballester-Caudet:** methodology, investigation, validation, writing-original draft. **R. Navarro-Utiel:** methodology, investigation. **I. Campos-Hernández:** methodology, investigation.

Declaration of Competing Interest

None.

Acknowledgment

Authors acknowledge EU FEDER and the Gobierno de España MCI-AEI (CTQ2017-90082-P, PID2021-124554NB-I00) and to the Generalitat Valenciana (PROMETEO Program 2020/078 and EU FEDER-Generalitat Valenciana (ID-FEDER/2018/049). A. B.-C. and R. N-U express their gratitude to Prometeo program for their post and pre-doc grants, respectively.

References

[1] M.R. Fowkes, Planetary forecast: the roots of sustainability in the radical art of the 1970s, Third Text 23 (2009) 669–674, doi:10.1080/09528820903189277.
[2] WCED (World Commission on Environment and Development)Our Common Future, Oxford University Press, Oxford, 1987.
[3] A. Gałuszka, Z. Migaszewski, J. Namieśnik, The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices, TrAC Trends Anal. Chem. 50 (2013) 78–84, doi:10.1016/j.trac.2013.04.010.

[4] M. Tobiszewski, M. Marć, A. Gałuszka, J. Namieśnik, Green chemistry metrics with special reference to green analytical chemistry, Molecules 20 (2015) 10928–10946, doi:10.3390/molecules200610928.
[5] I.T. Horváth, Introduction: sustainable chemistry, Chem. Rev. 118 (2018) 369–371, doi:10.1021/acs.chemrev.7b00721.
[6] P.M. Nowak, P. Kościelniak, What color is your method? Adaptation of the RGB additive color model to analytical method evaluation, Anal. Chem. 91 (2019) 10343–10352, doi:10.1021/acs.analchem.9b01872.
[7] A. Gałuszka, Z.M. Migaszewski, P. Konieczka, J. Namieśnik, Analytical eco-scale for assessing the greenness of analytical procedures, TrAC Trends Anal. Chem. 37 (2012) 61–72, doi:10.1016/j.trac.2012.03.013.
[8] J. Płotka-Wasylka, A new tool for the evaluation of the analytical procedure: green analytical procedure index, Talanta 181 (2018) 204–209, doi:10.1016/j.talanta.2018.01.013.
[9] A. Ballester-Caudet, P. Campíns-Falcó, B. Pérez, R. Sancho, M. Lorente, G. Sastre, C. González, A new tool for evaluating and/or selecting analytical methods: summarizing the information in a hexagon, TrAC Trends Anal. Chem. 118 (2019) 538–547, doi:10.1016/j.trac.2019.06.015.
[10] P.M. Nowak, P. Kościelniak, M. Tobiszewski, A. Ballester-Caudet, P. Campíns-Falcó, Overview of the three multicriteria approaches applied to a global assessment of analytical methods, TrAC Trends Anal. Chem. 133 (2020) 116065, doi:10.1016/j.trac.2020.116065.
[11] P.M. Nowak, R. Wietecha-Posłuszny, J. Pawliszyn, White analytical chemistry: an approach to reconcile the principles of green analytical chemistry and functionality, Trends Anal. Chem. 138 (2021) 116223, doi:10.1016/j.trac.2021.116223.
[12] M. Sajid, J. Płotka-Wasylka, Green analytical chemistry metrics: a review, Talanta 238 (2022) 123046, doi:10.1016/j.talanta.2021.123046.
[13] K. Parvathi, K. Abimanyu, S. Hemanth, K. Chanduluru, S. Rathinam, Environmental impact of greenness assessment tools in liquid chromatography – A review, Microchem. J. 170 (2021) 106685, doi:10.1016/j.microc.2021.106685.
[14] M.M. AbdelrahmanR.N., Green analytical chemistry metrics and life-cycle assessment approach to analytical method development, in: M.H. El-Maghrabey, V. Sivasankar, R.N. El-Shaheny (Eds.), Green Chemical Analysis and Sample Preparations. Chapter 2, Springer, Cham, Switzerland, 2022, pp. 29–99, doi:10.1007/978-3-030-96534-1_2.
[15] E.C. Vidotti, J.C. Cancino, C.C. Oliveira, M. do C.E. Rollemberg, Simultaneous determination of food dyes by first derivative spectrophotometry with sorption onto polyurethane foam, Anal. Sci. 21 (2005) 149–153, doi:10.2116/analsci.21.149.
[16] K. Yamjala, S.N. Meyyanathan, N.R. Ramisetti, Methods for the analysis of azo dyes employed in food industry-a review, Food Chem. 192 (2016) 813–824, doi:10.1016/j.foodchem.2015.07.085.
[17] Rovina K, S. Shafiquzzaman, S. Md Shaarani, A review of extraction and analytical methods for the determination of tartrazine (E 102) in foodstuffs, Crit. Rev. Anal. Chem. 47 (2017) 309–324, doi:10.1080/10408347.2017.1287558.
[18] J.F. Robens, G.S. Dill, J.M. Ward, J.R. Joiner, R.A. Griesemer, J.F. Douglas, Thirteen-week subchronic toxicity studies of Direct Blue 6, Direct Black 38, and Direct Brown 95 dyes, Toxicol. Appl. Pharmacol. 54 (1980) 431–442, doi:10.1016/0041-008X(80)90170-2.
[19] R.D. Combes, R.B. Haveland-Smith, A review of the genotoxicity of food, drug and cosmetic colours and other azo, triphenylmethane and xanthene dyes, Mutat. Res. Genet. Toxicol. 98 (1982) 101–243, doi:10.1016/0165-1110(82)90015-X.
[20] https://www.efsa.europa.eu/(Last access April 2022), (n.d.).
[21] T. Zou, P. He, A. Yasen, Z. Li, Determination of seven synthetic dyes in animal feeds and meat by high performance liquid chromatography with diode array and tandem mass detectors, Food Chem. 138 (2013) 1742–1748, doi:10.1016/j.foodchem.2012.11.084.
[22] M. Iammarino, A. Mentana, D. Centonze, C. Palermo, M. Mangiacotti, A.E. Chiaravalle, Dye use in fresh meat preparations and meat products: a survey by a validated method based on HPLC-UV-diode array detection as a contribution to risk assessment, Int. J. Food Sci. Technol. 55 (2020) 1126–1135, doi:10.1111/ijfs.14275.
[23] Y. Moliner-Martinez, A. Ballester-Caudet, J. Verdú-Andrés, R. Herráez-Hernández, C. Molins-Legua, P. Campíns-Falcó, in: In-Tube Solid Phase Microextraction in Handbooks in Separation Science, Solid Phase Extration edited by Colin F. Poole, 14, Elsevier, Amsterdam, 2019, pp. 275–317, doi:10.1016/B978-0-12-816906-3.00014-5. ChapterISBN: 978-0-12-816906-3.
[24] Y. Moliner-Martinez, R. Herráez-Hernández, J. Verdu-Andres, C. Molins-Legua, P. Campíns-Falcó, Recent advances of in-tube solid phase microextraction, TRAC 71 (2015) 205–213, doi:10.1016/j.trac.2015.02.020.
[25] M.E. Costa Queiroz, I. Donizeti de Souza, C. Marchioni, Current advances and applications of in-tube solid-phase microextraction, TRAC 111 (2019) 261–278, doi:10.1016/j.trac.2018.12.018.
[26] P. Serra-Mora, Y. Moliner-Martínez, C. Molins-Legua, R. Herráez-Hernández, J. Verdú-Andrés, P. Campíns-Falcó, Trends in online intube solid phase microextraction, Compr. Anal. Chem. (2017) 427–461, doi:10.1016/bs.coac.2017.01.002.
[27] K.S. Minioti, C.F. Sakellariou, N.S. Thomaidis, Determination of 13 synthetic food colorants in water-soluble foods by reversed-phase high-performance liquid chromatography coupled with diode-array detector, Anal. Chim. Acta. 583 (2007) 103–110, doi:10.1016/j.aca.2006.10.002.