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IDENTIFYING SYNTHETIC COLORANTS FROM WINE BY UPLC

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Abstract

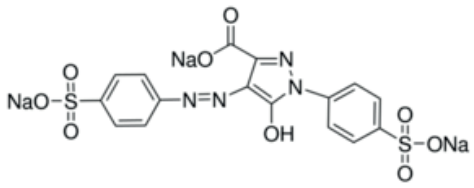
Legislation in force about wine quality states that concealing defects and alterations of the wines by introducing into their natural content of something that could determines changes in natural composition, aroma and taste could be a counterfeit product. This constitutes fraud and shall be punished according to the law. Synthetic colorants are a kind of additives, which although they are forbidden, may be found in wines. Consumption of them may affect the health of consumers with numerous side effects and toxicity, at both medium and long-terms, allergic reactions, behavioral and neurocognitive effects. To reduce consumers inconveniences and to avoid fraud in the wine sector, sensible analytical methods are required. Identification and quantification of some commonly used synthetic colorants (tartrazine - E102, amaranth - E123, sunset yellow- E110 and erythrosine - E127) is presented in this paper by ultra-performance liquid chromatography (UPLC) with UV detection in an adapted method for wine matrix. The method proves all of specific parameters for validation.

Key words*: food safety, quality, synthetic colorants, UPLC, wine.*

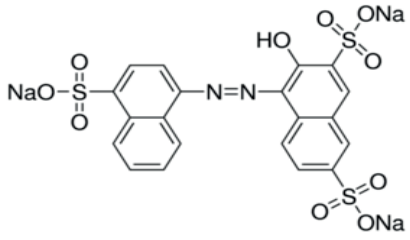
INTRODUCTION

The food colour is strongly associated with consumer choices (Zeece, 2020). Colour is the first important intrinsic sensory to consumers expectations regarding the flavour and taste of food and beverages. Natural alternatives are increasingly important for replaceing artificial colorants all over the world (Vinha et al., 2018). Using synthetic colorants in food becomes a major issue and this is the reason because food producers are trying to satisfy today's consumer demands with natural, and safe food products (Gebhardt et al., 2020). Consumers have more and more demands for natural plant-derived alternatives and also there are several scientific reports about harmfulness of synthetic colorants in food. (Vinha et al., 2018). Although there are currently allowed established acceptable daily intake (ADI) for food colorants and are very used, however there have been gradually substituted by those from natural origins. A lots of side effects and toxicity, at both medium and long-terms, behavioral and neurocognitive effects and allergic reactions have been related with their consumption. Otherwise, naturally-derived food colorants proves high quality, efficiency and organoleptic properties, and also have an important role as health promoters (Martins et al., 2016). Colorants are widely used in the food industry for improving food quality and food safety during processing, packaging and storage. Sourcing of these molecules is mainly done by three means: extraction from natural sources, chemical synthesis and bioproduction, the first two being the most utilized (Sun et al., 2021). A sensitive dispersive solid-phase extraction (D-SPE) method for the extraction and enrichment of four important synthetic colorants using high performance liquid chromatography was introduced by Chai W. et al in 2016, when the limits of detection (LODs) for the established d-SPE-HPLC method were 0.20-0.25 $\mu g\ L^{-1}$, which were lower than other chromatographic methods earlier reported for amaranth, ponceau 4R, sunset yellow and allure red. The method was also successfully applied to determination of colorants in samples of beverage with satisfactory results. Also, a new MSPE-HPLC method was developed for simultaneous determination of four synthetic colorants (amaranth, ponceau 4R, sunset yellow and allure red) in food samples (candy, jelly and carbonated drink) by Chen et al in 2019. The MSPE-HPLC method was simple and effective and can be used for the analysis of colorants in real samples.

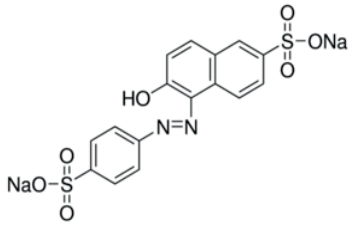
The most commonly synthetic colorants in food are: tartrazine or yellow acid 23 (E102), amaranth (E123), sunset yellow (E110) and erythrosine (E127) (Figure 1).



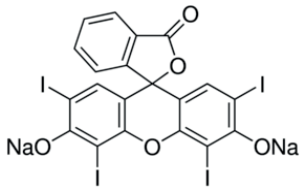
Tartrazine



Amaranth



Sunset Yellow



Erythrosine

Figure 1. Chemical structure of the four artificial colorants analysed in this study

The aim of present study was to find a reliable UPLC-UV method for analysing tartrazine, amaranth, sunset yellow and erythrosine in wine and to validate it. Assessed method parameters were: selectivity, linearity, sensitivity, accuracy, repeatability, reproducibility, limits of detection, limits of quantification, linear range and recoveries. After validation, the method was used to analyse the four artificial colorants in 20 wine samples.

MATERIALS AND METHODS

Materials

For this study the analytical standards of tartrazine (TZ) (trisodium (4E)-5-oxo-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)hydrazono]-3-pyrazolecarboxylate), amaranth (AM) (trisodium (4E)-3-oxo-4-[(4-sulfonato-1naphthyl)hydrazono]naphthalene-2,7disulfonate), sunset yellow (SY) (disodium 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonate) and erythrosine (ER) (acid 2-(6-hidroxi-2,4,5,7-tetraiodo-3-oxo-xanten-9-il)benzoic) were purchased from Dr. Ehrenstorfer. Ammonium formate (100%) were supplied by Thermo Fisher, acetonitrile (99.99%) and methanol (99.98%) were purchased from Honeywell and the ethanol (99.9%) were purchased from Merck, all of them being HPLC grade. Samples of bottled wine were purchased from local markets and were classified as red, rosé and white wines and also as dry, medium dry, medium sweet and sweet. The wine samples were stored at 4-8°C until analysis.

Standard solutions and sample preparation

Stock standard solutions were prepared with 1000 mg/L concentration in ultapure water for each of analytical standard. Then from these stock solutions was prepared a mixed working standard solution containing 100 mg/L TZ, 100 mg/L AM, 100 mg/L SY and 100 mg/L ER in 10% methanol. For obtaining the calibration curve were prepared five standard levels by diluting the mixed working standard solution with 10% methanol. Stock standard solutions and mixed working standard solution were stored at 4-8°C prior to use.

Wine samples have removed it's possible gases by sonication, then were properly diluted with ultrapure water. After dilution stage, the samples were pH adjusted to 6.5 ± 0.1, filtered using nonsterile hydrophobic PTFE syringe filter with 0.45 µm pore size and placed in an UPLC vial for instrumental analysis.

The fortified test samples were prepared by spiking at the LOQ level, respectively at 5 mg/L TZ, 5 mg/L AM, 5 mg/L SY and 5 mg/L ER.

Chromatographic method

For identifying and quantifying the four synthetic colorants in wine samples by liquid chromatography method, it was used Waters Acquity UPLC equipment (with binary solvent manager, thermostatic column compartment, heater/cooler sample organizer) with UV detector. The separation was performed with a Kinetex EVO C18 column from Phenomenex (1.7 µm, 100Å, 150 mm x 2.1 mm), at 40°C, by isocratic elution with 0.2 mL/min flow rate. The mobile phase was 10 mM ammonium formate prepared in ultrapure water. All solvents were sonicated before using. The injection volume was setted at 1.3 µL and the run time at 15 minutes. Our synthetic colorants detection was performed at 420 nm wavelength for yellow compounds, like tartrazine and sunset yellow and at 530 nm wavelength for red ones, like amaranth and erythrosine. Data were collected and processed using Empower 2 software.

RESULTS AND DISCUSSIONS

Presented chromatographic method was applied for identification and quantification of the four synthetic colorants. After diluting stock solutions stage, each analyte was injected in turn for determining their sequence. All the four artificial colorants were identified in the obtained chromatogram for the mixed standard solution, in the following order: at 420 nm wavelength TZ (2.26 min retention time) and SY (5.11 min retention time) and at 530 nm wavelength AM (3.82 min retention time) and ER (7.25 min retention time). The analysis proved a good separation of all four compounds to each wavelength, with resolution and symmetry and also peaks shape (Figures 2.1. and 2.2.). Retention times determinated and peak width proved that resolution values were calculated at 2.5 for TZ, 3.8 for SY, 4.3 for AM and 4.5 for ER.

The purpose of the method was validation it for demonstrating that its performance characteristics are adequate (Barwick et al., 2014). There were setted and confirmed all of specific validation parameters like selectivity, limit of detection and limit of quantification, working range, trueness, analytical sensitivity, precision, ruggedness, uncertainty measurement (Barwick et al., 2014).

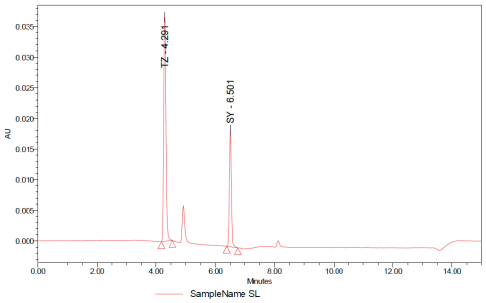


Figure 2.1. Chromatogram of mixed standard solution (SL) at 420 nm wavelength

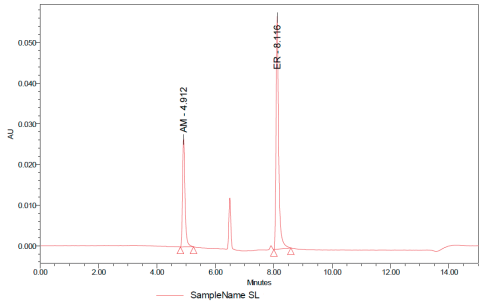


Figure 2.2. Chromatogram of mixed standard solution (SL) at 530 nm wavelength

Selectivity is about a method's ability to highlight the differences of analysis determination in presence of other compounds that could be similarly (Rusea, 2016). The peaks were without overlapping, so each compound were individual registered, there wasn't interferences and it was a clear identification of them (Figures 2.1. and 2.2.).

Limit of detection (LOD) is the minimum amount of analyte that could be detected by equipment in a test sample, but this couldn't be quantified as an exact number value (Rusea, 2016). In this study it was evaluated a signal-to-noise ratio of 3 for LOD.

LOD were determined at following values: 0.034 mg/L for TZ, 0.049 mg/L for AM, 0.031 for SY and 0.072 mg/L for ER.

Limit of quantification (LOQ) is the minimum amount of analyte that could be quantitatively determined by equipment in a test sample with acceptable accuracy and repeatability (Rusea, 2016) and is about 10 x LOD. LOQ were determined at following values: 0.34 mg/L for TZ, 0.49 mg/L for AM, 0.31 for SY and 0.72 mg/L for ER.

Working range is the interval between minimum and maximum concentration of an analyte in a test sample (Rusea, 2016). In our study the working range was setted from 0.25 mg/L to 20.0 mg/L for TZ, AM and SY and from 1.0 mg/L to 20.0 mg/L for ER.

Linearity is when we have an established domain and the method proves the ability to provide a set of results that are directly proportional to analyte concentration value (Rusea, 2016). External calibration method was performed for quantitative analyzes. Calibration curve was performed with standard solutions with five concentration levels, with three injections per each level. Correlation coefficient was higher than 0.999 for all of four synthetic studied colorants. The registered values of r^2 were 0.999985 for TZ, 0.999998 for AM, 0,999966 for SY and 0.997139 for ER.

Analytical sensitivity is the modification of measuring instrument response reported to the stimulus changing (Rusea, 2016). It is when an analytical method proves minimum concentration variations of an analyte and our method proved it.

Accuracy or trueness of an analytical method is about how close to the true value is the determinated value. It express the raport between average value of analytical set results and a reference value that is accepted (Rusea, 2016). Bias is the expressed term for measuring accuracy and establishes a total systematic error. Our study proves a bias of 1.95% for TZ, 9.93 for AM, 1.74 for SY and 19.72 for ER.

Precision or *repeatability* is determined by a series of analytical determinations obtained from different aliquots of the same test sample, in same conditions (Rusea, 2016). This is a part of measurement uncertainty and is expressed as relative standard deviation (RSD%). Repeatability was established by six times injecting in a row three levels of the mixed standard solution. RSD was assessed to the following averages values: 4.87% for TZ, 6.3% for AM, 4.03% for SY and 6.46% for ER.

Reproductibility is when repeatability could be made by another analyst using the same analytical procedure for determining the same test samples or by the same analyst but with another, similar, equipment. The reproducibility in our study was assessed by analysing the same three levels of mixed standard solution, 6 times in a row by two analysts. RSD average values were following: 5.11% for TZ, 6.01% for AM, 4.95% for SY and 9.54% for ER.

Recovery is a determination of method efficiency for detecting all quantity of analyte, it is express by percentage and is about the real concentration of studied recovered substance during the analyzes (Rusea D., 2016). Recovery is a rapport between extracted samples response, obtained for three analyte concentrations, and response without the extraction stage. Our test samples ware fortified to the LOQ level, respectively with 5 mg/L of each colorant and registered recovery values were 96% for TZ, 101% for AM, 109% for SY and 93% for ER.

Measurement uncertainty is a technical parameter that could be associated with the measurement result that is associated with attributed values dispersion to the measurement. Measurement uncertainty involves to evaluate of the errors sources at each analytical stage and estimation of associated uncertainty. The global uncertainty means to have available datas, quality control and comparison tests.(Rusea, 2016). We setted a measurement uncertainty up to 8% for this analytical method.

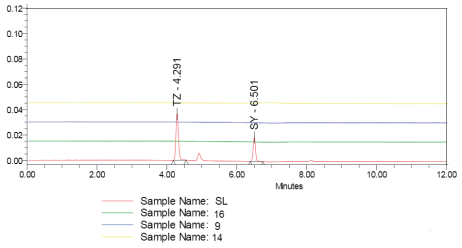


Figure 3.1. Overlay chromatogram of three negative wine samples for tartrazine and sunset yellow (at 420 nm wavelength) and mixed standard solution (SL)

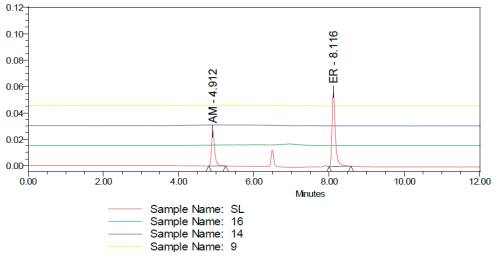


Figure 3.2. Overlay chromatogram of three negative wine samples for amaranth and erythrosine (at 530 nm wavelength) and mixed standard solution (SL)

The presented analytical method was used for assessing the possible presence and the quantify the four studied synthetic colorants in 20 different bottled wine samples from Romanian market. Although, Virtanen et al. observed in 1999 that erythrosine prepicitates when is added into wine, we can not confirm this, but also we admit that we didn't do a stability study for the addition of erythrosine in wine to be able to observe any possible precipitate in time. Assessing the obtained data, there weren't identified any of the four synthetic colorants in the analized wine samples. (Ex. Figure 3.1. and 3.2.). Tartrazine, amaranth, sunset yellow and erythrosine were not detected in any sample. We identified slight traces of amaranth in a single bottled wine sample but the value was below of LOQ and this means that it was not possible to quantified it.

CONCLUSIONS

The UPLC-UV method proves that is suitable for identifying and determining of the concentration of tartrazine, amaranth, sunset yellow and erythrosine that could be illegally added in wine. The analytical method is rather simple, with no need of special sample preparation. The reliability of this method is assured by the obtained values for the validation parameters.

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