**Revista de Chimie**
*https://revistadechimie.ro*
*https://doi.org/10.37358/Rev. Chim.1949*

# Environmental Detection of Brilliant Blue, Sunset Yellow and Tartrazine Using Direct Injection HPLC-DAD Technique

**FLORINELA PIRVU[1,2], VASILE ION IANCU[1], MARCELA NICULESCU[1], CAROL BLAZIU LEHR[1], LUOANA FLORENTINA PASCU[1], TOMA GALAON[1]***
[1]National Research and Development Institute for Industrial Ecology – ECOIND, 71-73 Drumul Podu Dambovitei Str., 060652, Bucharest, Romania
[2]University Politehnica of Bucharest, Faculty of Biotechnical Systems Engineering, 313 Splaiul Independentei, 060042, Bucharest, Romania

***Abstract:*** *A newly developed direct injection HPLC-DAD method for the simultaneous detection of Brilliant Blue (BB), Sunset Yellow (SY) and Tartrazine (TA) synthetic dyes was validated and applied to surface and wastewater samples. Chromatographic separation was achieved using an Acclaim Surfactant Plus column (150 x 3.0 mm, 3 um) in isocratic elution mode with a mobile phase consisting of 100 mM $CH_3COONH_4$ and ACN in the ratio 50/50 (v/v). Linear calibration curves were plotted in the range of 2-100 µg/L (BB) and 6-300 µg/L (SY, TA) respectively with correlation coefficients ($R^2$) higher than 0.99. Low quantitation limit values (LOQ) were obtained for the direct injection HPLC-DAD method, namely: 1.0 µg/L (BB), 5.5 µg/L (SY) and 5.8 µg/L (TA), respectively. The entire sample preparation procedure consisted only in simple filtration using PTFE syringe filters (0.45 µm) without sample extraction or concentration, which improved method accuracy and precision. HPLC-DAD developed method proved to be fast (6 minutes run-time), sensitive and accurate and was succesfully applied for the analysis of the synthetic dyes in surface and wastewater samples. Considering all analyzed samples (8 surface water and 11 wastewater samples), Brilliant Blue was found in influent samples with values between 4.49 ÷ 14.17 µg/L and effluent samples with values between 1.07 ÷ 9.54 µg/L, whereas Sunset Yellow and Tartrazine were not detected in any of the analyzed samples.*

***Keywords****: Brilliant Blue, Sunset Yellow, Tartrazine, HPLC-DAD, direct injection, surface water, wastewater*

## 1. Introduction

Environmental pollution caused by the frequent use of dyes is one of the main problems in developed countries. This is due to improper use of natural resources and poor compliance with the legislation. There have been a number of scientific studies for the development and implementation of new treatment technologies and even ecological actions [1-4].

A current problem for aquatic ecosystems that can cause a change in the natural balance of flora and fauna is the discharge of industrial and domestic wastewater loaded with various xenobiotic dyes into natural receptors usually represented by rivers and lakes [5]. Among the industrial waters involved are those that come from activities that use dyes in their production cycles. Therefore, we are talking about food factories, textile companies, distilleries, cosmetics manufacturers, pharmaceutical and chemical factories, paper factories, printing companies or refineries [6]. Synthetic dyes are a non-nutritional, chemically active component that confers secondary coloration or pigmentation to food. The dyes are made up of chromogenic groups, and the most well-known is certainly that of the nitrogen dyes. For example, it falls into this category: Tartrazine, Sunset Yellow. Most food additives have at least one azo group (double bond N = N). The dyes, especially the nitrogenous ones, have complex aromatic structures. Precisely for this reason, their degradation does not take place effectively with conventional biological treatment methods. Therefore, different treatments are needed to discolor wastewater. Currently, special attention is paid to the determination of food colorant contents. Some of them, especially when are consumed in large quantities, present a potential risk to human health [7].

**email: email: tomagalaon@yahoo.com*

The yellow dye, Auramine, affects the growth of the kidneys and liver. The use of Rhodamine can lead to the breakdown of red blood cells. Yellow methanil dye affects the reproductive organs, but it can also cause the stomach and kidney to degenerate. The use in large quantities of Amaranth and Tartrazine can cause DNA damage in the glands of the stomach, colon and bladder. These can trigger unwanted effects, which can cause allergies, asthma and attention deficit hyperactivity disorder (ADHD) [8]. The use in high amounts of synthetic dyes can cause internal organ damage, as well as carcinogenic diseases [9].

The spectrum of reactions induced by food additives ranges from benign manifestations of the disease, such as redness or rhinorrhea, to life-threatening symptoms such as anaphylaxis and anaphylactic shock with allergic responses usually ranging from a few minutes to 2 hours [10]. Allergies, hyperactivity, irritability, aggressiveness and learning deficiencies are related to food coloring. Recurrent urticaria, angioedema or dermatitis may be caused by Ponceau 4R dye and among 25 children aged between 1.5 and 12.5 years have reported allergic reactions after the intake of food dye. Sunset yellow is used mainly in the food and pharmaceutical industries [11].

Blood and bone marrow neoplasm, such as non-Hodgkin's lymphoma, is a form of cancer of the lymphatic system, which may occur in people who use hair dyes that contain dyes of the type: parafenylenediamine (PFD), para-toluenediamnine (PTD), para-aminophenol (PAP). PFD poisoning has been reported in humans and mammals, which can lead to acute respiratory disorders, rhabdomyolysis, and optic nerve atrophy [12,13]. In studies conducted on children, following the use of the products that have the composition of the colorant Amaranth (chewing gum, sweet sauces without fruit content, semi-prepared pasta containing), hives and aggravation of atopic dermatitis have been observed [14]. Among different types of food additives, food colorants play an important role in food because of their physical appearance.

The use of synthetic dyes is preferred because it offers a color intensity and uniformity, they are stable, they are easily homogenized in the manufacturing processes and they are less expensive [15]. The wastewater matrices are complex and different from the physical-chemical point of view and the content of colorings makes it difficult to develop an adequate analytical method. To perform a multi-residue analysis with high efficiency and high sensitivity, it is important to develop a new and efficient method for determination and quantification of dyes in different environmental water matrices.

The aim of this study was to develop a fast, sensitive, accurate and cost-efficient HPLC-DAD method for the simultaneous determination of three synthetic dyes from complex environmental samples (surface and wastewater). Finally, a new HPLC-DAD method with direct injection (20 µL) of environmental samples without any sample extraction, concentration and purification was developed and validated. No concentration step was required which improved significantly method accuracy and precision.

## 2. Materials and methods

### 2.1. Chemicals and working standard solution

High purity analytical standards were used as follows: Brilliant Blue (purity ≥ 97.0%), Sunset Yellow, (purity ≥ 95.0%), Tartrazine (purity ≥ 99.0%) were obtained from Sigma-Aldrich (Steinheim, Germany). Acetonitrile, Methanol and Ammonium Acetate for liquid chromatographic analysis (HPLC grade) were purchased from Merck (Darmstadt, Germany). Chromafil XTRA PTFE type filters with a pore size of 0.45 µm and a diameter of 25 mm used for samples filtration were produced at Macherey-Nagel. The stock standard solutions with a concentration of approximately 1000 mg/L were prepared in a mixture of methanol / water (50:50). Individual dilutions and mixed analyte standard solutions were prepared in ultrapure water.

### 2.2. Apparatus and chromatographic method

The experiments to determine the optimum conditions for chromatographic separation and detection were performed on an Agilent 1200 series HPLC system equipped with: semipermeable

membrane degasser; quaternary pump; autosampler with variable injection volume (0.1-100 µl); thermostatted column compartment and a Diode Array Detector (DAD) with the ability to record simultaneously on-line UV-Vis spectra (190-900 nm) and up to 8 discrete wavelengths in this range. The Agilent ChemStation software was used for data acquisition, processing and reporting. Considering that the simultaneous determination of three compounds was attempted, the conditions for their chromatographic separation were realized in isocratic mode. Different compositions of the mobile phase were tested using different proportions of Acetonitrile and Ammonium Acetate. The optimum mobile phase composition determined experimentally was 100 mM Ammonium acetate in ultrapure water with a pH value of 5.0 (solvent A) and Acetonitrile (solvent B) = 50/50 (v/v). All chromatographic runs were carried out on a Acclaim Surfactant Plus column (150 x 3.0 mm, 3.0 µm) from Thermo Scientific which was kept at 30°C. Experiments were performed in isocratic elution conditions at a flow rate of 0.6 mL/min. Injection volume was 20 µL and the chromatogram run time was only 6 minutes.

The detection of the target compounds was performed at specific wavelengths corresponding to at the optimum wavelengths identified after the maximum absorption from UV-VIS spectra at 428 nm for Tartrazine, 484 nm for Sunset Yellow, and the additive Brilliant Blue had a maximum absorption at 628 nm was determined. Figures 1, 2 and 3 show the structural formulas and absorption spectra for each dye: Brilliant Blue, Sunset Yellow, Tartrazine.

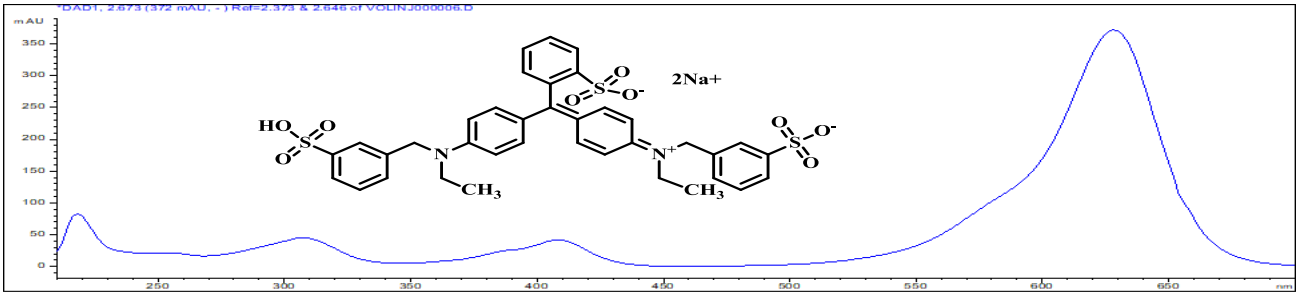


**Figure 1**. Structural formula of Brilliant Blue FCF (E133) and corresponding UV absorption spectrum (210 – 700 nm); absorption maximum used for detection at 628 nm

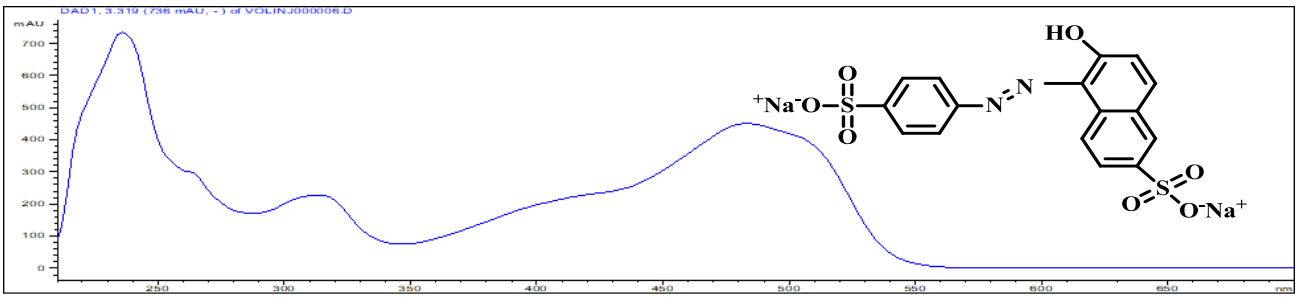


**Figure 2**. Structural formula of Sunset Yellow (E110) and corresponding UV absorption spectrum (210 – 700 nm); absorption maximum used for detection at 484 nm

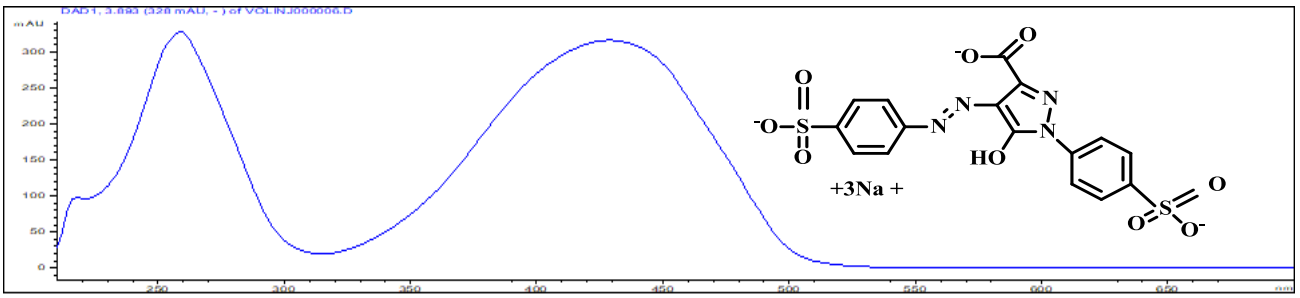


**Figure 3**. Structural formula of Tartrazine (E102) and corresponding UV absorption spectrum (210 – 700 nm); absorption maximum used for detection at 428 nm

# 3. Results and discussions

## 3.1. Optimization of separation and detection parameters

Considering the significant polarity and water solubility of all three target compounds (Brilliant Blue - log Kow = -4.94, solubility 30 g/L; Sunset Yellow - log Kow = -1.18, solubility 190 g/L; Tartrazine - log Kow = -10.17, solubility 200 g/L) which are mainly given by the sulfonic groups present in their structures, it was decided to separate these compounds using a chromatographic column (Acclaim Surfactant Plus) usually dedicated for surfactants which also contain fully ionized groups polar moieties ($SO_3^-$, $NH4^+$ and so on) [16].

To obtain a better separation, the column temperature was modified between 20 and 40°C (5°C steps), while for the verification of method sensitivity injection volume was increased from 2 to 100 µL. The temperature of 30°C and the injection volume of 20 µL proved to be the optimum values for highest chromatographic resolution (Rs), the increased peak efficiency (N) and the shortest run-time (only 6 min) and with maximum sensitivity.

For the elution under isocratic regime different compositions of the mobile phase were tested using different proportions between the aqueous phase and the organic solvent (50/50, 52/48, 55/45, 60/40). The optimum composition was 50% aqueous phase (100 mM ammonium acetate pH 5) and 50% organic solvent (Acetonitrile), which resulted in narrow peaks with high peak efficiency. The optimized conditions of the liquid-chromatographic parameters allowed the separation of the three analytes in a chromatographic run-time of only 6 minutes. To optimize these parameters, a solution of Brilliant Blue, Sunset Yellow and Tartrazine with a concentration of 50 µg/L, 150 µg/L and 150 µg/L respectively, was used. In Figure 4 is represented the variation of retention time with column temperature change in the range 20 - 40°C. As expected, temperature increase (20 - 40°C) leads to analyte retention time decrease. As predicted by van't Hoff equation this decrease is generally linear. Indeed, correlation coefficients for the retention time – temperature dependence was higher than 0.99 for all 3 analytes. Nonetheless, the decrease is not drastic and no coelution appears even at 40°C.

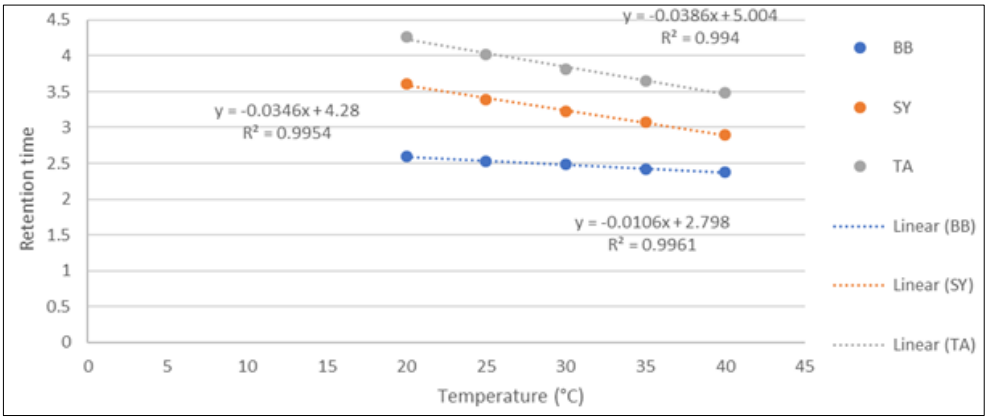


**Figure 4**. Linear decrease of retention under the influence of temperature increase (20-40°C) for Brilliant Blue (BB), Sunset Yellow (SY), Tartrazine (TA)

The final chosen column temperature was 30°C because at this value peak width and peak efficiency were greater than at 20°C which allowed higher resolution but also increased column pressure and run-time.

Method injection volume was optimized in the range 2 - 100 µL by considering its effect on chromatographic parameters: peak width, peak symmetry and peak area. Final method injection volume was chosen at 20 µL because it offered a compromise between: maximum sensitivity (high values of peak area and noise-signal-ratio), low peak widths, maximum resolution between BB-SY and SY-TA peak pairs, and last but not least the interference limitation from the complex matrix of wastewater samples injected directly into HPLC. Injection volume of 20 µL allowed very low quantitation limits for the target analytes (1.0 µg/L for BB, 5.5 µg/L for SY and 5.8 µg/L for TA).

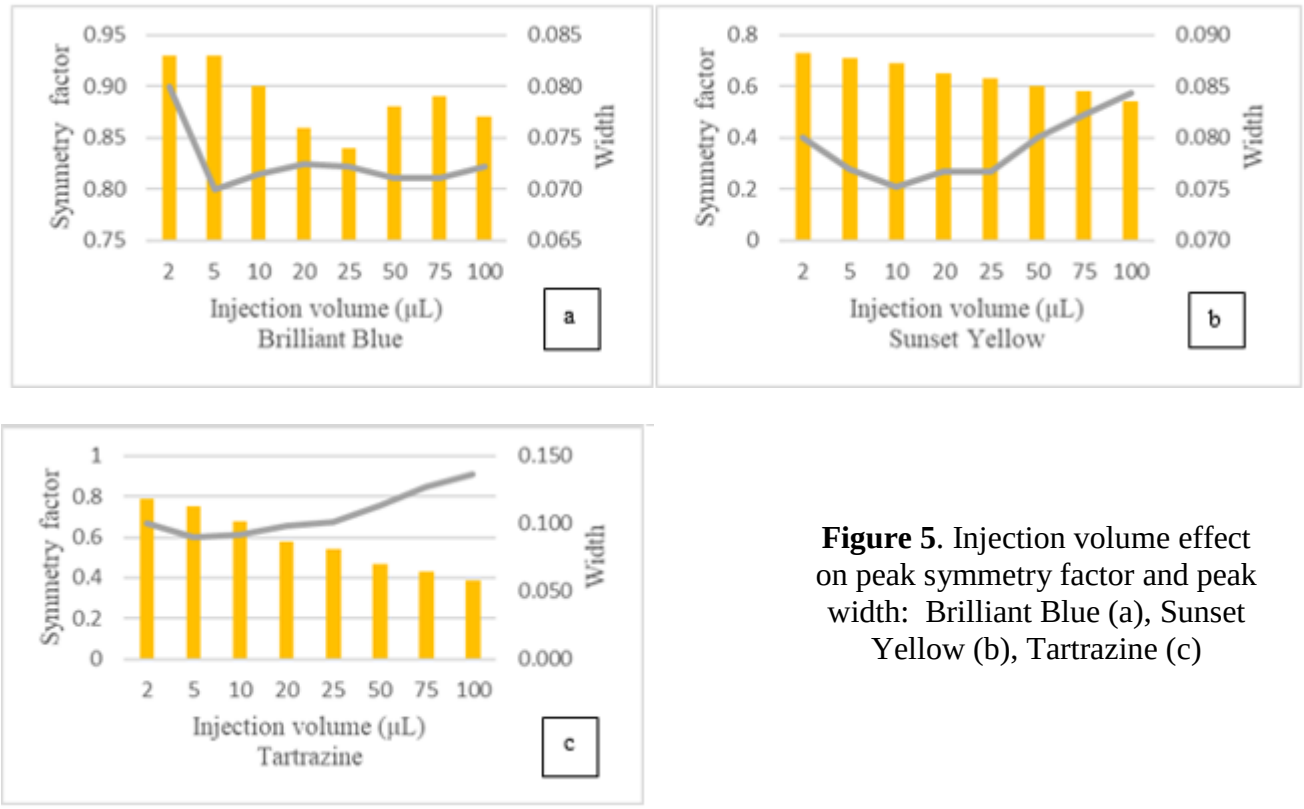


**Figure 5**. Injection volume effect on peak symmetry factor and peak width: Brilliant Blue (a), Sunset Yellow (b), Tartrazine (c)

Figure 5 depicts the influence of the injection volume on the symmetry factor and on the peak width of the chromatographic peaks corresponding to target analytes. For SY and TA it can be observed that an injection volume higher than 20 µL generates a decrease in peak symmetry and an increase in peak width. In the case of BB, peak width is rather constant for injection volumes between 20 and 100 µL which is also valid for symmetry factor. These observations support also the choice of 20 µL injection volume.

Following the LC detection optimization procedure, the parameters which generated maximum sensitivity (minimum peak width, maximum efficiency, maximum signal to noise ratio) were chosen for all compounds analyzed in conjunction with a minimum duration of chromatographic separation. Following the optimization of the parameters, the quantitation limits of the method (LOQ) for the three analytes were determined [17]. LOQ quantitation limit for each of the 3 dyes (µg/L) was determined by injecting decreasing concentrations of standard solutions of BB, SY, TA until a signal-to-noise ratio value of 10 was obtained.

### 3.2. Environmental matrix removal by simple filtration

Laboratory experiments on the filtration of environmental samples (surface water and wastewater) were performed in order to establish the optimal procedure of these compounds and the elimination of potential interferences. The process of separating analytes from aqueous media using syringe filters is increasingly used in environmental analysis due to its advantages: it eliminates the consumption of toxic organic solvents for the environment, avoids the use of high capacity laboratory glassware and working time is reduced.

The effluent samples from 2 municipal wastewater treatment plants were taken in brown glass containers filled in such way that the amount of air present in the container is as small as possible. These were transported in refrigerated boxes and were pre-treated within 24 h. The samples were first analyzed as such, without the addition of a standard to observe the eventual presence of any target compound in the water sample. The recovery of the analytes from the water samples was performed for concentrations of 10 µg/L Brilliant Blue, 30 µg/L Sunset Yellow, 30 µg/L Tartrazine and

respectively for concentrations of 50 µg/L Brilliant Blue, 150 µg/L Sunset Yellow, 150 µg/L Tartrazine, and the results are shown in Table 1.

**Table 1.** Recovery yields calculated after sample filtration on 0.45 µm PTFE filters.

| Analyte | Recovery (%) | | |
|---|---|---|---|
| | 10 µg/L BB, 30 µg/L SS, 30 µg/L TA | 50 µg/L BB, 150 µg/L SS, 150 µg/L TA | Average (%) |
| Brilliant Blue | 92.9 | 95.7 | 94.3 |
| Sunset Yellow | 90.7 | 91.9 | 91.3 |
| Tartrazine | 93.6 | 97.2 | 95.4 |

Recovery values obtained after filtration of effluent samples spiked with the three analytes at 2 concentration levels were higher than 90%. These values were averaged to determine the retrieval efficiency of BB, SY and TA by filtering on PTFE filters of 0.45 µm (BB 94.3%, SY 91.3%, and TA 95.4%) (Figure 6).

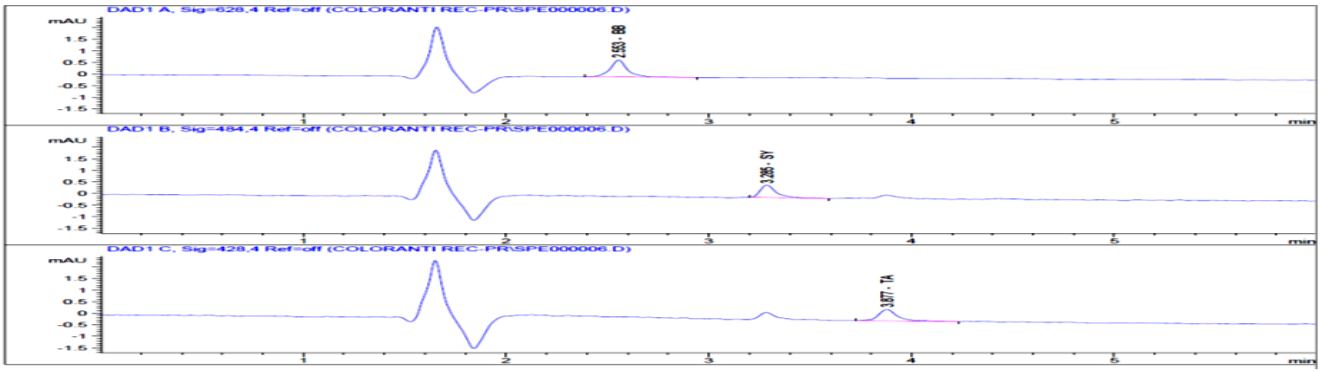


**Figure 6**. Chromatogram of an effluent sample spiked with a mixture of 10 µg/L Brilliant Blue (BB), 30 µg/L Sunset Yellow (SY), 30 µg/L Tartrazine (TA)

The results showed that the matrix of the water samples discharged from the 2 treatment plants, but also the filtration of the samples on PTFE type filters have a minimal effect (<10%) on the determination of the compounds of interest.

### 3.3. Method validation

The developed HPLC-DAD method for the detection of the 3 target analytes from surface and wastewater samples was fully validated. Linearity was evaluated by analyzing five standard solutions for each dye. As shown in Table 2, for all compounds the correlation coefficients were higher than 0.99.

**Table 2.** Linear range, regression equations and correlation coefficents for calibration curves

| Analyte | Linear range | Regression equation | $R^2$ |
|---|---|---|---|
| Brilliant Blue | 2–100 µg/L | y= 0.17712x + 0.51483 | 0.9911 |
| Sunset Yellow | 6–300 µg/L | y = 0.05523x + 0.59705 | 0.9946 |
| Tartrazine | 6–300 µg/L | y = 0.05410x + 0.41518 | 0.9932 |

The selectivity of the method was evaluated by injecting a standard solution containing the three dyes, a sample of wastewater that does not contain interest analytes and a sample of ultrapure water

(blank). Considering that at the wavelengths of interest there were no interfering peaks at the retention times corresponding to the 3 dyes, it can be considered that the method is selective. This can be seen from the overlay of chromatograms presented below (Figure 7, Figure 8 and Figure 9).

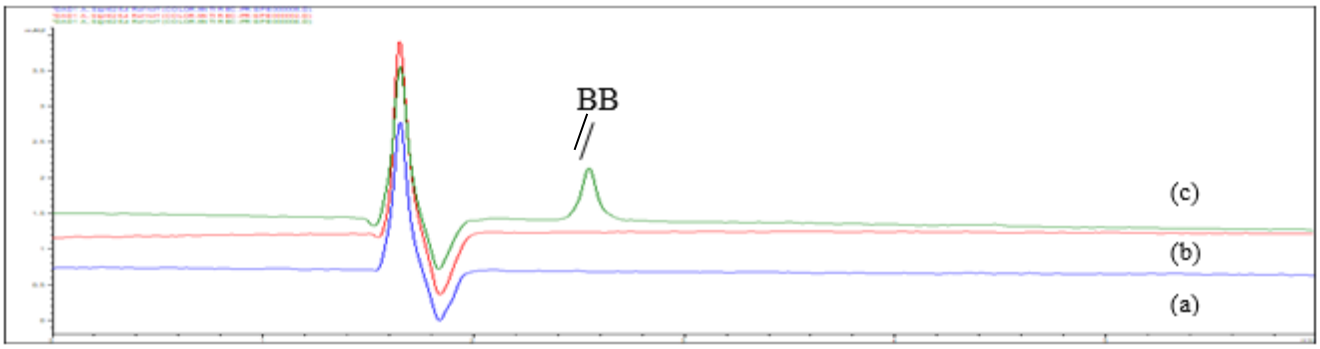


**Figure 7**. Overlaid chromatograms corresponding to injection of: Blank (a), Wastewater (b) and Standard (c) at λ = 628 nm (Brilliant Blue)

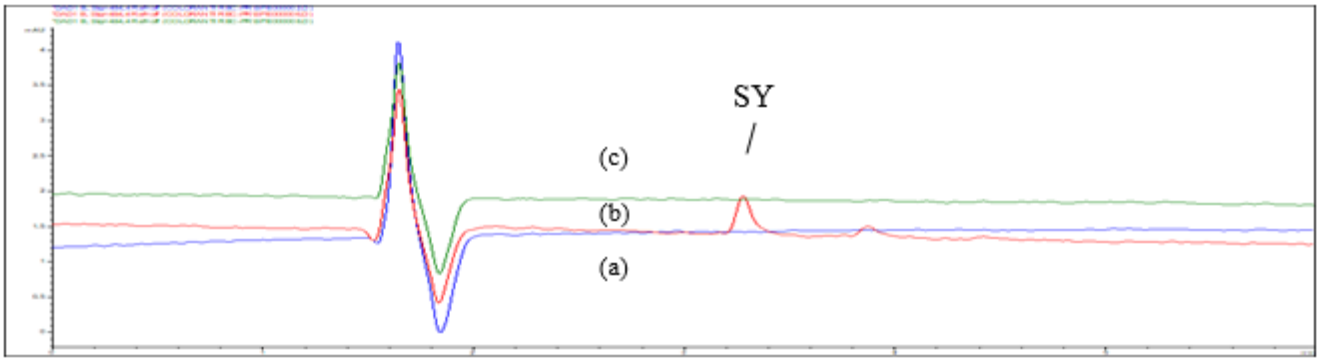


**Figure 8**. Overlaid chromatograms corresponding to injection of: Blank (a), Standard (b) and Wastewater (c) at λ = 484 nm (Sunset Yellow)

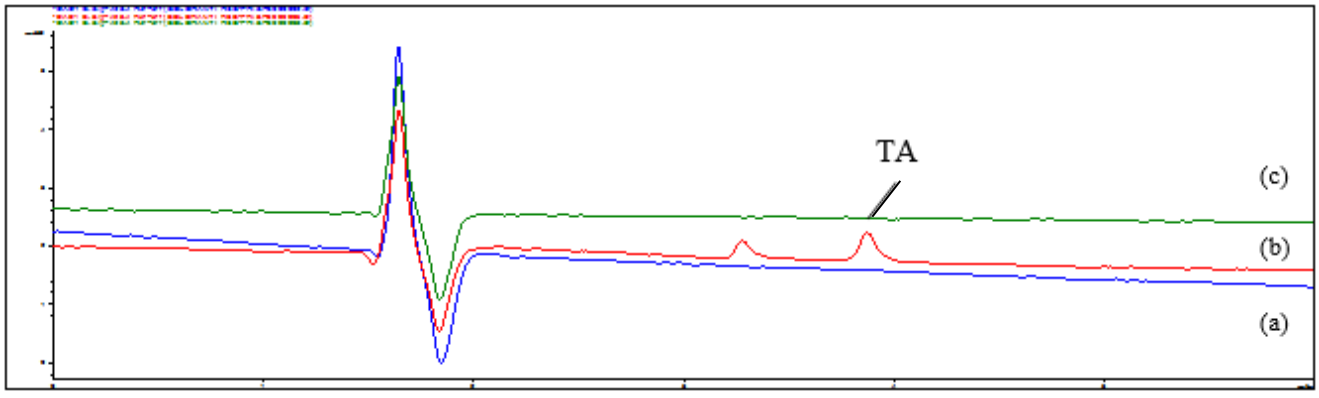


**Figure 9**. Overlaid chromatograms corresponding to injection of: Blank (a), Standard (b) and Wastewater (c) at λ = 428 nm (Tartrazine)

The method repeatability was evaluated by repeating the procedure for preparing and analyzing the samples on 6 sub-tests from the same sample. Method reproducibility was evaluated by applying the sample preparation procedure on a single sample divided into 12 sub-tests, but which were prepared on different days by several analysts. The precision of the entire analytical procedure, expressed as relative percentage standard deviation (RSD%), was determined by repeated analysis of effluent samples of wastewater treatment plants having the concentrations presented in Table 3. The precision of the method varied for repeated measurements below 10%. The results are presented in Table 3, and in Figures 10, 11, 12, the overlapping chromatograms of the repeatability test for 50 µg/L BB, 150

µg/L SY and 150 µg/L TA are shown.

**Table 3.** RSD% values obtained for method precision

| Analyte | Concentrations µg/L | Repeatability (RSD%) (n=6) | Reproducibility (RSD%) (n=12) |
|---|---|---|---|
| Brilliant Blue | 50 | 3.8 | 7.4 |
| | 100 | 1.1 | 2.6 |
| Sunset Yellow | 150 | 3.3 | 7.4 |
| | 300 | 1.2 | 3.2 |
| Tartrazine | 150 | 4.0 | 7.0 |
| | 300 | 1.3 | 3.7 |

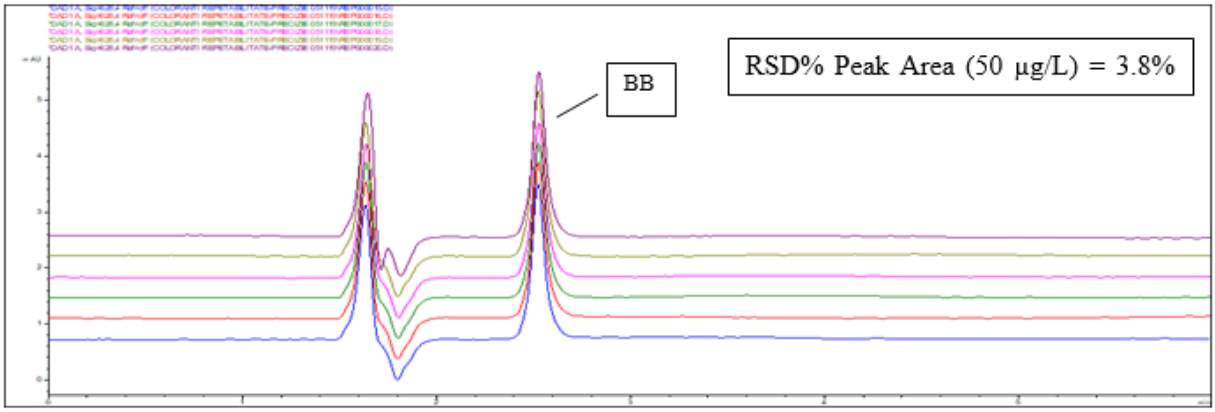


**Figure 10**. Chromatogram of the repeatability test for the Brilliant Blue compound at 50 µg/L

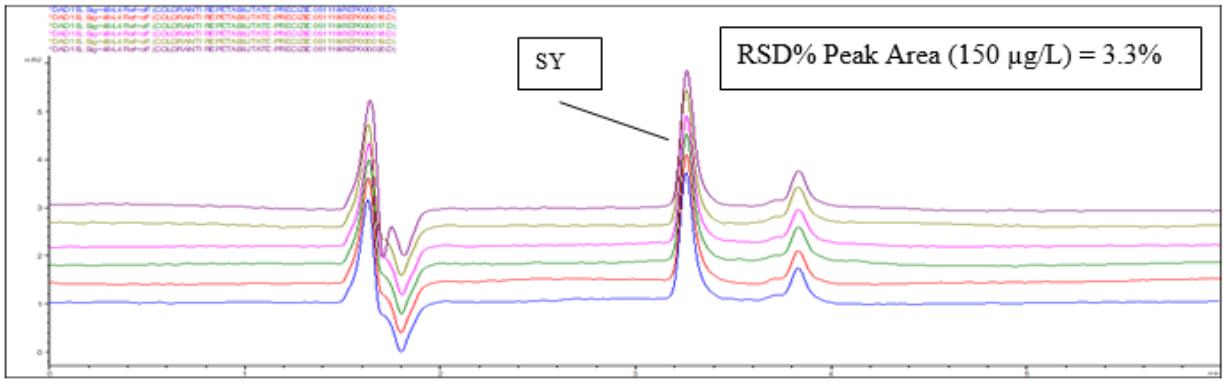


**Figure 11**. Chromatogram of the repeatability test for the Sunset Yellow compound at 150 µg/L

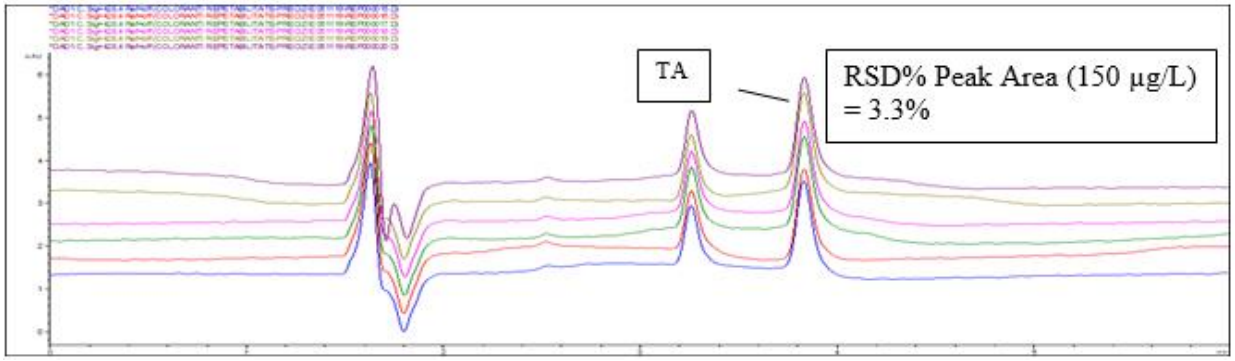


**Figure 12**. Chromatogram of the repeatability test for the Tartrazine compound at 150 µg/L

The detection (LOD) and quantification (LOQ) limits of the method were determined by injecting

solutions with increasingly lower analyte concentrations until the experimentally determined signal-to-noise ratio was equal to 3 (LOD) and 10 (LOQ), respectively. The values of the detection and quantification limits are presented in table 4.

**Table 4**. Method detection and quantitation limits (LOD, LOQ).

| Analyte | LOD (µg/L) | LOQ (µg/L) |
|---|---|---|
| Brilliant Blue | 0.30 | 1.0 |
| Sunset Yellow | 1.65 | 5.5 |
| Tartrazine | 1.74 | 5.8 |

### 3.4. Occurrence of synthetic dyes in surface and waste water samples

For the determination of target synthetic dyes (Brilliant Blue, Sunset Yellow, Tartrazine) by direct injection method, several wastewater and surface water samples were subjected to analysis. The samples were filtered through Chromafil XTRA PTFE syringe filters with a pore size of 0.45 µm and a diameter of 25 mm. An injection volume of 20 µL was used. The applicability of the method was tested on 19 wastewater and surface samples (4 samples taken upstream (AM), 4 samples taken downstream (AV), 2 samples of influent (INF) and 9 samples of effluent (EF)). From the chromatographic analysis of surface water and wastewater samples taken from the area of a city in the north-western part of Romania (AM, INF, EF, AV), it was observed a decrease in BB concentration values in the order: P4 INF (14.17 µg/L) > P5 EF (5.21 µg/L)> P6 AV (<LOD). Presence of the same BB dye in wastewater from the entry and exit of the wastewater treatment plant of another city in the east of Romania with very close values, namely P8 INF (4.49 µg/L) > P9 EF (4.37 µg/L) > P10 AV (<LOD) shows that this treatment plant only allows a reduced removal of this dye. From the chromatographic analysis of the wastewater samples taken from the outflow from the treatment plants (effluent) of some localities in the center of Romania, it can be observed that the additive BB has a concentration value in descending order, as follows: P14 EF (9.54 µg/L) > P13 EF (3.14 µg/L) > P18 EF (2.69 µg/L) > P15 EF (2.29 µg/L) > P16 EF (2.24 µg/L) > P17 EF (1.83 µg L) > P19 EF (1.07 µg/L). In all 19 samples of wastewater and surface water analyzed, the other two additives, namely Sunset Yellow and Tartrazine, are below the quantitation limit (<LOQ), namely 5.5 µg/L for SY and 5.8 µg/L for TA. Results for all analyzed samples are given in Table 5.

**Table 5**. Occurrence of synthetic dyes in surface and waste water samples

| Samples | Conc. BB (µg/L) | Conc. SY (µg/L) | Conc. TA (µg/L) |
|---|---|---|---|
| P1 AM | < LOQ | < LOQ | < LOQ |
| P2 AV | < LOQ | < LOQ | < LOQ |
| P3 AM | < LOQ | < LOQ | < LOQ |
| P4 INF | 14.17 | < LOQ | < LOQ |
| P5 EF | 5.21 | < LOQ | < LOQ |
| P6 AV | < LOQ | < LOQ | < LOQ |
| P7 AM | < LOQ | < LOQ | < LOQ |
| P8 INF | 4.49 | < LOQ | < LOQ |
| P9 EF | 4.37 | < LOQ | < LOQ |
| P10 AV | < LOQ | < LOQ | < LOQ |
| P11 AM | < LOQ | < LOQ | < LOQ |
| P12 AV | < LOQ | < LOQ | < LOQ |
| P13 EF | 3.14 | < LOQ | < LOQ |
| P14 EF | 9.54 | < LOQ | < LOQ |
| P15 EF | 2.29 | < LOQ | < LOQ |
| P16 EF | 2.24 | < LOQ | < LOQ |
| P17 EF | 1.83 | < LOQ | < LOQ |
| P18 EF | 2.69 | < LOQ | < LOQ |

| Samples | Conc. BB (µg/L) | Conc. SY (µg/L) | Conc. TA (µg/L) |
|---|---|---|---|
| P19 EF | 1.07 | < LOQ | < LOQ |

## 4. Conclusions

In this study a newly developed direct injection HPLC-DAD method for the simultaneous detection of Brilliant Blue (BB), Sunset Yellow (SY) and Tartrazine (TA) synthetic dyes was validated and applied to surface and wastewater samples. No sample extraction was applied since method sensitivity and intrinsic detector selectivity did not require it. Chromatographic separation was achieved in only six minutes. Sample preparation was readily obtained using simple PTFE filtration on 0.45 um syringe filters.

All LC parameters (nature and composition of the mobile phase, injection volume, flow-rate, column temperature and detector wavelength) were optimized for the rapid separation of the three analytes with high sensitivity in order to determine these synthetic dyes at concentration levels of parts per billion (µg/L) from complex matrices of wastewater and surface water. Target analyte recovery (method accuracy) for the developed HPLC-DAD method was 94.3% for BB, 91.3% for SY and 95.4% for TA, which are fully acceptable values for the low tested concentration levels. Regarding the precision of the direct injection method, RSD values were obtained between (1.1% - 4.0%) in the case of repeatability and between (2.6% - 7.4%) in the case of the intermediate precision. The obtained LOQ values were 1.0 µg/L for Brilliant Blue, 5.5 µg/L for Sunset Yellow and 5.8 µg/L for Tartrazine respectively. These low values for an HPLC method coupled with DAD detection were mainly due to the UV-Vis chromophore-rich structures of the analytes that have intense absorption bands (high molar absorptivity coefficients) in the visible range. Generally, the visible range is free of majority of interferences from organic compounds present in waste and surface waters. Simple filtration of environmental samples followed by direct injection HPLC-DAD analysis allowed for a significant reduction in analysis time and an increase in method accuracy and precision.

The developed method was successfully applied to determine the dyes from 11 wastewater samples and 8 surface water samples collected from Romania. Brilliant Blue was detected in waste water at concentrations levels ranging between 4.49 µg/L and 14.17 µg/L for influents, and at concentrations levels sitated in the range 1.07 ÷ 9.54 µg/L for effluent samples. Considering surface water, the samples taken upstream and downstream of the treatment plant, the concentration of Brilliant Blue was below LOQ. With respect to Sunset Yellow and Tartrazine, none was detected in any of the analyzed samples (<LOQ).

**Acknowledgments**: The authors acknowledge the financial support provided by the Ministry of Research and Innovation through the national research programme "Nucleu", contract number 20N/2019, Project code PN 19 04 01 01.

**References**

1. PIECZYŃSKA, A., OSSOWSKI, T., BOGDANOWICZ, R., & SIEDLECKA, E., Electrochemical degradation of textile dyes in a flow reactor: effect of operating conditions and dyes chemical structure, *Int. J. Environ. Sci. Technol*., **16**, 2019, 929-942.
2. MOHAN, S.V., BHASKAR, Y.V., KARTHIKENYAN, J., Biological decolourization of simulated azo dye in aqueous phase by algae Spirogyra species, *Int. J. Environ. Pollut*. **21** (3), 2004, 211-222.
3.VAGHELA, S.S., JETHVA, A.D., MEHTA, B.B., DAVE, S.P., ADIMURTHY, S., RAMACHANDRAIAH, G., Laboratory studies of electrochemical treatment of industry azo dye effluent, *Environ. Sci. Technol*., **39** (8), 2005, 2848-2855.
4. CARDOSO, J.C., LIZIER, T.M., ZANONI, M.V.B., Highly ordered TiO2 nanotube arrays and photoelectrocatalytic oxidation of aromatic amine. *Appl. Catal. B Environ*., **99** (1-2), 2010, 96-102.
5. CHIRIAC, F.L., PAUN, I., PIRVU, F., PASCU, L.F., GALAON, T., Liquid Chromatography Tandem Mass Spectrometry Method for Ultra-Trace Analysis of Organic UV Filters in Environmental

Water Samples, *Rev. Chim*., **71** (1), 2020, 92-99.
6. COLLIVIGNARELLIA, M.C., ABBÀB, A., MIINOA, M.C., DAMIANIA, S., Treatments for color removal from wastewater: State of the art, *J. Environ. Manage*., **236**, 2019, 727-745.
7. FENG, J., CERNIGLIA, C.E., CHEN, H., Toxicological significance of azo dye metabolism by human in-testinal microbiota, *Front. Biosci*., **4**, 2012, 568–586.
8. ZAHRA, N., KALIM, I., SAEED, M.K., MUMTAZ, Z., AMJAD, N., ALIM-UN-NISA, HINA, S., MASOOD, S., AHMED I., AND ASHAF M., Effect of natural and synthetic dyes on human health, *Int. J. Biol. Sci*., **6**(10), 2017, 23-29.
9. GOLKA, K., KOPPS, S., MYSLIAK, Z.W., Carcinogenicity of azo colorants: influence of solubility and bioavailability, *Toxicol. Lett*., **151**, 2004, 203-210.
10. RONA, R. J., KEIL, T., SUMMERS, C., GISLASON, D., ZUIDMEER, L., SODERGREN, E., SIGURDARDOTTIR, S.T, LINDNER, T., GOLDHAHN, K., DAHLSTROM, J., McBRIDE, D., MADSEN, C., The prevalence of food allergy: a meta-analysis. *J. Allergy Clin. Immun.*, **120**, 2007, 638–646.
11. FEKETEA G., TSABOURI S., Common food colorants and allergic reactions in children: Myth or reality?, *Food Chemistry*, **230**, 2017, 578-588.
12. NOHYNEK, G.J., FAUTZ, R., KIEFFER, F.B., TOUTAIN, H., Toxicity and human health risk of hair dyes, *Food Chem. Toxicol.*, **42**, 2004, 517-543.
13. IWEGBUE C.M.A., BASSEY, F.I., OBI, G., TESI, G.O., MARTINCIGH, B.S., Concentrations and exposure risks of some metals in facial cosmetics in Nigeria, *Toxicol. Rep*., **3**, 2016, 464-472.
14. CARREON, T., HEIN, M.J., VIET, M.J., HANLEY, K.W., RUDER, A.M., WARD, E., Increased bladder cancer risk among workers exposed to o-toluidine and aniline: a reanalysis, *Occup. Environ. Med.*, **67** (5), 2010, 348–350.
15. KANAREK, R.B., Artificial food dyes and attention deficit hyperactivity disorder. *Nutr. Rev*, **69**(7), 2011, 385-391.
16. PAUN, I., IANCU, V.I., CRUCERU, L., NICULESCU, M., CHIRIAC, F.L., Simultaneous Determination of Anionic and Cationic Surfactants Mixtures in Surface Water, *Rev. Chim*., **69**(1) 2018, 27-30.
17. CHIRIAC, F.L., PAUN, I., PIRVU, F., CRUCERU, L., PASCU, L.F., GALAON, T., Parallel between offline-SPE-LC-MS and direct injection LC-MS methods for Acrylamide detection in drinking water at parts per trillion level", *Rev. Chim*., **69** (11), 2018, 4129-4133.

Manuscript received 08.04.2020