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Simultaneous Determination by HPLC-UV Vis of Tartrazine and Sunset Yellow in Soft Drinks Sold in Benin

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Abstract

The use of dyes such as tartrazine (E102) and sunset yellow (E102) in food, beverages and health products for technological and commercial purposes is common. The adverse effects caused by these dyes, such as allergies and hyperactivity disorder have been reported, especially in children. In the present study, a chromatographic method was developed and validated for simultaneous determination of tartrazine and sunset yellow. The chromatographic separation was performed on a Lichrocart® C18 column (125 × 4.6 mm; 5 µm) with a security Guard-C_{18} column (4 × 2.0 mm, 5 µm; Phenomenex, Torrance, CA, USA) maintained at 30˚C. The mobile phase consisted of a mixture of acetonitrile/ammonium acetate buffer pH 6.8 in gradient mode with a flow rate of 1 mL/min. The injection volume was 10 µL. The detection wavelength was set at 455 nm. The parameters of specificity, linearity, precision, repeatability, accuracy and sensitivity were examined for validation. The developed method is linear in the range of 1 µg/mL to 100 µg/mL with a $R^2 >$ 0.998. The intra-day and inter-day precisions (RSD) were less than 0.6% and 3.1% respectively. The detection limit was 0.03 µg/mL and the quantification limit was 0.1 µg/mL. The retention time of tartrazine was 2.86 min, while sunset yellow was detected at 5.67 min. A simple, rapid, accurate and robust HPLC/UV-Visible method was developed and validated for simultaneous identification and quantification of tartrazine and sunset yellow. This developed method was successfully applied for the simultaneous determination of

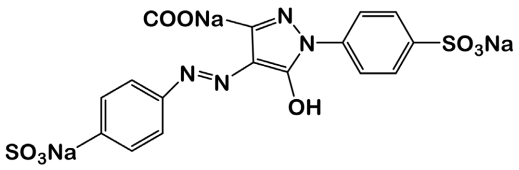
tartrazine and sunset yellow in soft drinks sold in Benin.

Keywords

Tartrazine, Sunset Yellow, HPLC-UV, Food Dyes

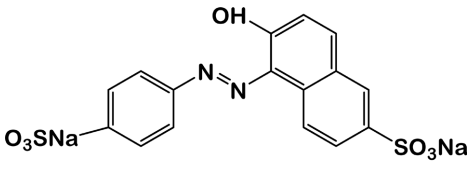
1. Introduction

The human body is essentially made up of water. Water represents 60-70% of the body mass of an adult. In total, the body needs 2 to 2.5 liters per day to function properly and this need is met through drinking water [1]. For reasons of taste, better organoleptic characteristics and pleasure, people have turned to non-alcoholic soft drinks such as lemonades and carbonated drinks to quench their thirst and meet the body's need for water [2]. Food colors are used in the manufacture of many soft drinks where there are intended to change the color of the beverages to make them more attractive to consumers and increase their consumption. A distinction is made between natural food dyes, which are beneficial to health, and synthetic chemical food dyes, which are the most commonly used but sometimes constitute a threat to human health [3] [4]. Among the most commonly used dyes in the soft drink industry are tartrazine (E102), chemically known as trisodium (4E)-5-oxo-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)hydrazono]-3-pyrazolecarboxylate, $C_{16}H_9N_4Na_3O_9S_2$ (**Figure 1**), and sunset yellow (E110), chemically known as disodium 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalnesulfonate, $C_{16}H_{10}N_2Na_2O_7S_2$, (**Figure 2**) [5]. They are found in several soft drinks with different flavors like lemon, pamplemousse, orange, tangerine, banana, fruit cocktail and so [6]. Studies have proven that high daily intake or long-term exposure to these food dyes could cause health issues with various toxicity manifestations such as allergies, genotoxicity and hyperactivity [7] [8] [9] [10]. In



E102

Figure 1. Structural representation of tartrazine.



E110

Figure 2. Structural representation of sunset yellow.

order to protect the population, food products manufactured with dyes are required for general safety such as acceptable daily intake (ADI) as well as proper labelling and identification by codes established by international regulatory bodies such as the Codex Alimentarius and the Food and Drug Administration (FDA) [11] [12]. Despite regulations, several food manufacturers like soft drinks manufacturers use food dyes without serious control in developing countries [13] [14]. The lack of serious quality control of soft drinks in those countries would expose populations to many health risks such as acute or chronic poisoning, and allergies due to food dyes contained in soft drinks and other foods [15] [16] [17]. Therefore, it is necessary to ensure the quality control of those products. Various methods for determination of azo food dyes such as tartrazine and sunset yellow have been developed. Spectrophotometric methods are commonly used; however, there may be limited by lack of specificity due to spectral overlap with other species in the matrix [13] [18]. Capillary Electrophoresis (CE) analyses are fast and economical while being efficient [19]. Unfortunately, most laboratories especially in Africa have less access to CE equipment. Moreover, a wide range of High Performance Liquid Chromatography (HPLC) coupled with UV-Visible, DAD or MS detectors have been used to analyse food dyes but required a complex elution gradient program, a detection system with different wavelengths, complex logarithmic assistance and long analysis time [20] [21]. Therefore, this study aims to develop a simple, selective and sensitive HPLC-UV visible method for simultaneous quantification of tartrazine and sunset yellow in soft drinks.

2. Experimental

2.1. Chemicals and Reagents

Tartrazine reference (98.8%) and sunset yellow reference (99.2%) used in this study were supplied by Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Acetonitrile HPLC grade was purchased from Merck KGaA (Darmstadt, Germany). Formic acid, and ammonium acetate were of analytical grade and purchased from VWR Chemicals (Leuven, Belgium). Milli-Q water obtained from the Milli-Q plus purification device (Millipore, Billerica, MA, USA) was used throughout the experiment.

2.2. Instrumentation and Chromatographic Condition

The liquid chromatography was performed using HPLC instrument, HITACHI VWR system (VWR internationnal, Pennsylvanie, USA), equipped with a binary pump HITACHI VWR 5160, an autosampler HITACHI VWR 5260, a temperature-controlled column compartment HITACHI VWR 5310 and a UV-DAD detector HITACHI VWR 5430. The separation of the analytes was achieved on a Lichrocart® C_{18} column (125 × 4.6 mm; 5 µm) with a security Guard-C_{18} column (4 × 2.0 mm, 5 µm; Phenomenex, Torrance, CA, USA). The mobile phase consisted of the mixture of acetonitrile and 10 mmol/L ammonium acetate buffer

pH 6.8 in gradient elution with a flow rate at 1 mL/min. The column temperature was kept at 30˚C and the autosampler temperature was maintained at 15˚C with an injection volume of 10 µL. The detection wavelength was set at 455 nm with an analysis time of 8 min. The gradient program was set for 10 min and displayed in Table 1. The system control and data acquisition were carried out by the Chromaster software version 1.1.

2.2.1. Preparation of Calibration Standard and Quality Control Samples

The stock solutions of tartrazine and sunset yellow were prepared in water to a concentration of 1 mg/mL and kept at 4˚C. The stock solutions were serially diluted to yield working solutions at different concentration levels from 2 µg/mL to 200 µg/mL for tartrazine and 2 µg/mL to 200 µg/mL for sunset yellow. Calibration samples were then prepared with appropriate amounts of working solutions. The concentrations of calibration standard were: 1.0, 3.0, 10.0, 30.0, 50.0 and 100.0 µg/mL for tartrazine and for sunset yellow. QC samples were prepared at three concentration levels of 3.0 µg/mL (low QC), 30.0 µg/mL (middle QC) and 50.0 µg/mL (high QC) for tartrazine and for sunset yellow.

2.2.2. Sample Preparation

A volume of 10 mL of the sample (soft drink) was taken in a 50 mL Erlenmeyer flask and degassed in an ultrasonic bath for 30 min until all gas bubbles disappeared. 2 mL of the degassed solution was taken and filtered through a 0.45 µm membrane filter. The filtered solution was then injected into the HPLC for analysis. Analysis was done in triplicate.

2.3. Method Validation

The method was validated according to the criteria developed by the "International Conference of Harmonization" [22] [23]. The parameters evaluated to assess the reliability of the results consisted of:

- Linearity of the chromatographic response as a function of analyte concentration from 1 - 100 µg/mL for tartrazine and for sunset yellow.
- Repeatability of the chromatographic analysis of tartrazine and sunset yellow solution at three (3) concentration levels: 3, 30 and 50 µg/mL (n = 6) respectively;
- Repeatability of chromatographic analysis of a sample of soda (n = 6);
- Repeatability of the procedure (n = 6);

Table 1. Gradient program.

Time (min)	Acetonitrile (%)	Buffer (%)
0	4	96
6	30	70
7	4	96
10	4	96

- Accuracy using the standard additions method. Different amounts of tartrazine and sunset yellow (3, 30 and 50 µg/ml) were added to a soda sample. The samples with or without the addition were subjected to chromatographic analysis to determine the percent of recovery;
- The limits of detection (signal-to-noise ratio of 3) and quantification (signal-to-noise ratio of 10) determined were assessed from serial dilutions of the standard solution of tartrazine and sunset yellow 1 µg/mL.

To assess carry-over effects, blanks were injected immediately after the highest concentration of the calibration standard (50.0 µg/mL) in order to check any interfering peak at the retention time of analytes.

2.4. Statistical Treatment

The linearities of the responses of tartrazine and sunset yellow were assessed from a scatter plot. The regression lines were determined according to the least square's method. An analysis of variance (ANOVA) was performed to test the statistical significance and the overall slope of the regression line. The repeatability or precision was assessed through the relative standard deviation (RSD) calculated from the ratio of the standard deviation to the mean of each series of measurements. The accuracy was assessed through the relative error (RE) of each series of measurements.

2.5. Application to Tartrazine and Sunset Yellow Determination in Soft Drink

The developed HPLC–UV visible method was applied for the simultaneous determination of tartrazine and sunset yellow in soda drink sold in Benin. In this study, eighteen (18) samples of soft drinks from six (6) brands made of three (3) different batches each and containing tartrazine and sunset yellow were analyzed. They were packaged in Polyethylene terephthalate (PET) bottles and manufactured in Benin or imported from Nigeria and sold in Benin. These carbonated dyes based beverages came from different manufacturers. The samples were coded as follows: samples EL-A, EL-B, EN-C; EN-D; EN-E and EN-F. Each sample was analyzed three (3) times under the fixed analysis conditions. The instrument and analysis condition were those described in section "2.2". The concentration of tartrazine and sunset yellow was compared with the recommended upper limit value of 100 µg/mL according to codex alimentarius.

3. Results

3.1. Method Validation

3.1.1. Selectivity and Carry-Over

The selectivity of the method was determined by analyzing blank controls. It was found that no interference appeared at the retention times of both tartrazine and sunset yellow which are 2.86 min and 5.67 min respectively. Typical chromatograms of tartrazine standard solution (**Figure 3**), sunset yellow standard solution

(Figure 4) and a mixture of tartrazine and sunset yellow standard solution (Figure 5), were shown, respectively. No carry-over peaks were detected for all the analytes in the chromatogram of blank injected after the highest concentration of calibration sample.

3.1.2. Linearity, Accuracy and Precision

The calibration curves were linear over the concentration range of 1 - 100 µg/mL with (R^2 = 0.9998) for tartrazine and 1 - 100 µg/mL with (R^2 = 0.9993) for sunset yellow. The typical equations of calibration curves were f = (9848 ± 95) × C +

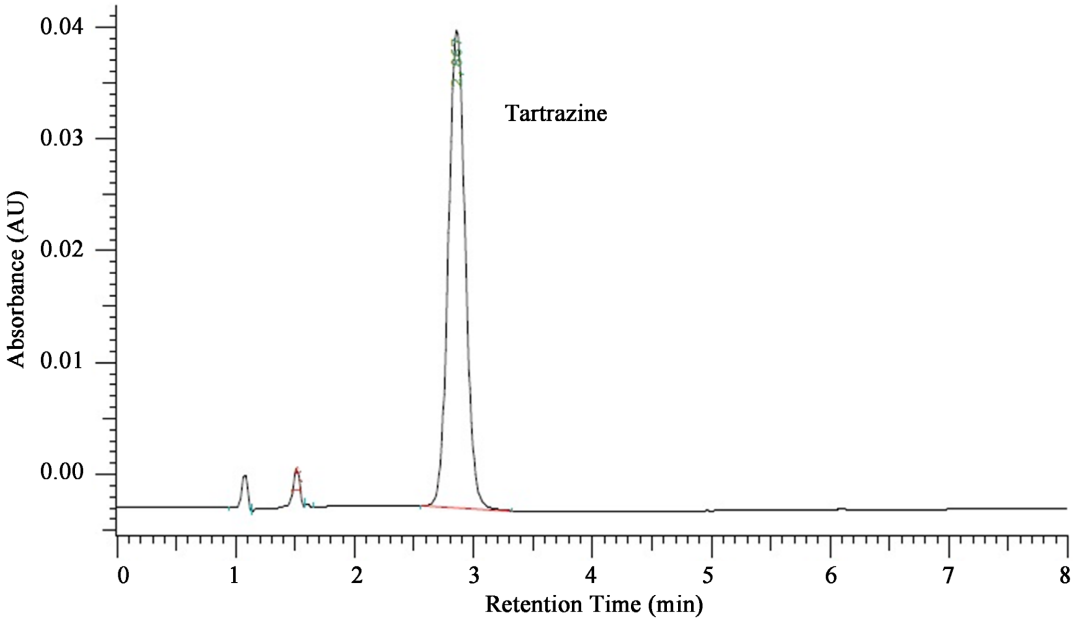


Figure 3. Typical chromatogram of tartrazine standard solution.

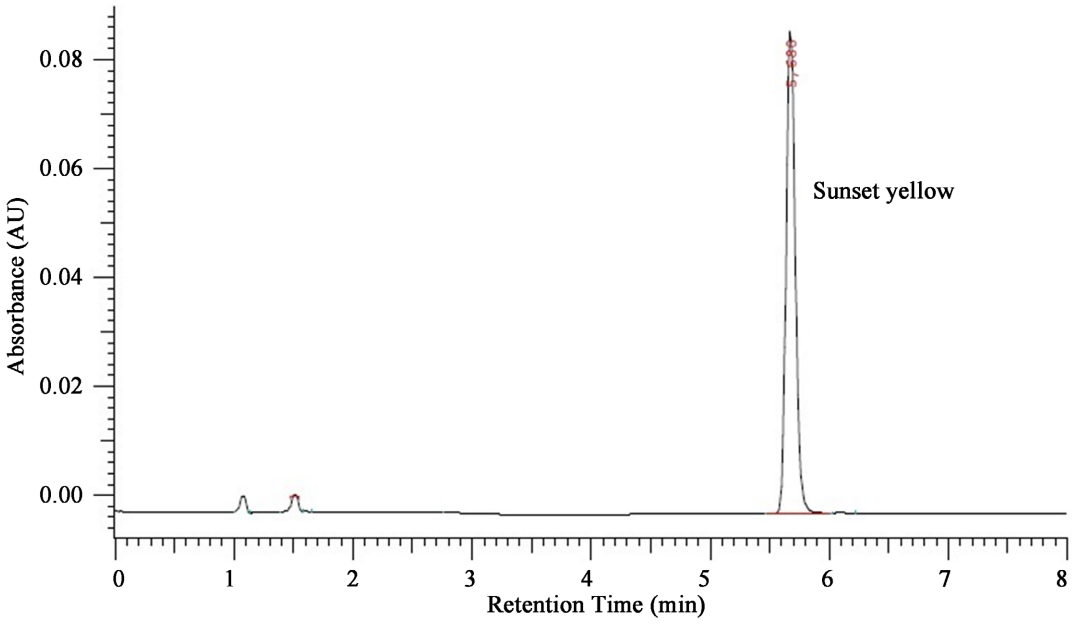


Figure 4. Typical chromatogram of sunset yellow standard solution.

(1600 ± 1387) for tartrazine and f = (10,146 ± 140) ×C + (2380 ± 1713) for sunset yellow, where f represents the chromatographic peak area of the analytes and C represents the concentration of the analytes.

The validation samples of six replicates of QC samples were prepared and analyzed in three separate analytical batches to evaluate the precision and accuracy of the method. The precision (RSD) and accuracy (RE) data for the analysis of tartrazine and sunset yellow in soft drinks were summarized in Table 2. The limits of detection and quantification were found to be 0.03 and 0.1 μg/mL for tartrazine and sunset yellow respectively. Dilution integrity were carried out when sample concentrations were higher than the upper limit of calibration curve. The accuracy and precision for diluted sample were within ±5%, suggesting that samples with concentration above upper limit of quantitation could be reanalyzed by appropriate dilution. The method was accurate and precise.

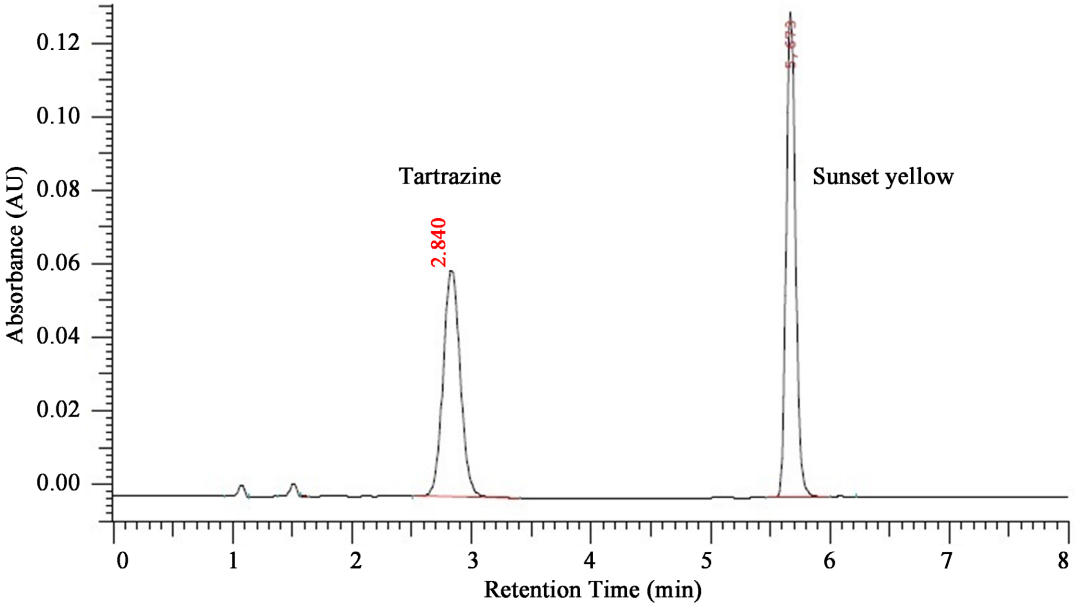


Figure 5. Typical chromatogram of mix tartrazine and sunset yellow standard solution.

Table 2. Precision (RSD) and accuracy (RE) data for the analysis of tartrazine and sunset yellow in soft drinks.

	Concentration levels (μg/mL)	Concentration added (μg/mL)	RSD (%)		RE (%) Accuracy
			Intra-day	Inter-day	
Tartrazine	Low QC	3	0.6	2.3	1.0
	Middle QC	30	0.2	1.9	0.7
	High QC	50	0.1	1.8	1.1
Sunset yellow	Low QC	3	0.5	3.1	−0.7
	Middle QC	30	0.2	2.6	0.7
	High QC	50	0.1	1.9	0.5

3.2. Quantification of Tartrazine and Sunset Yellow in Soft Drink

The proposed method was successfully applied for simultaneous identification and quantification of tartrazine and sunset yellow in soft drink sold in Benin. Figure 6 shows the chromatogram from the analysis of a soft drink sample. The concentration of tartrazine and sunset yellow in soft drink samples were calculated using the calibration lines. Table 3 shows the results of the simultaneous identification and quantification of tartrazine and sunset yellow in soft drink samples by HPLC_UV Visible. The Concentrations of tartrazine range from 2.84 to 21.59 µg/mL while the concentrations of sunset yellow range from 3, 05 to 73.99 µg/mL.

4. Discussion

The various methods for the identification and determination of azo food dyes such as tartrazine and sunset yellow have been developed. Spectrophotometric methods are commonly used; however, there may be limited by lack of specificity

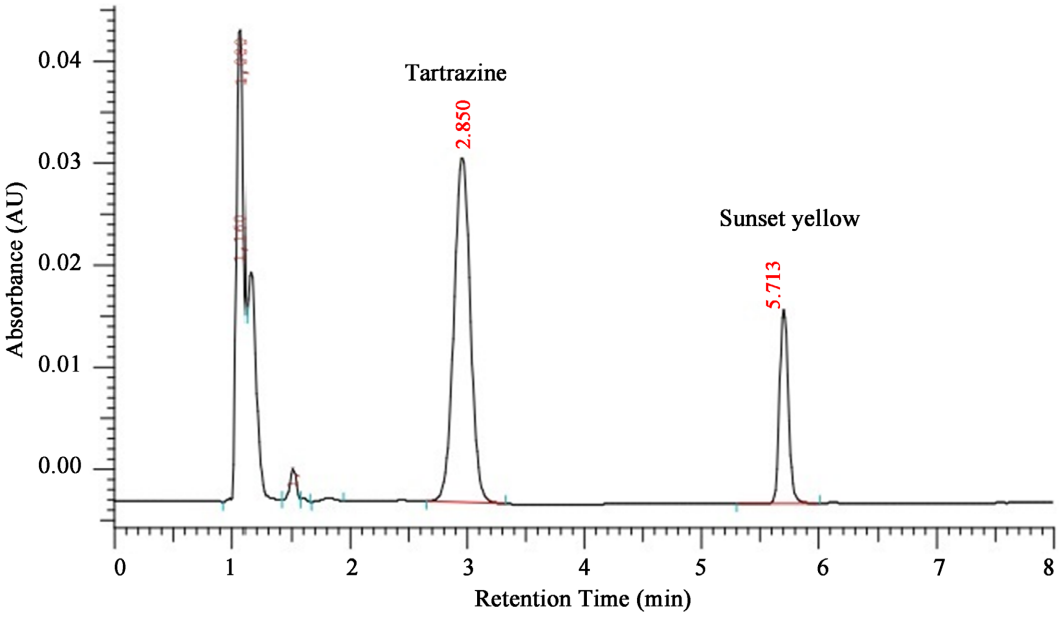


Figure 6. Chromatogram from the analysis of a soft drink sample.

Table 3. Determination of tartrazine and sunset yellow in soft drink samples.

Samples	Tartrazine concentration (µg/mL)	Sunset yellow concentration (µg/mL)	Acceptance criteria	Results
EL-A	13.73 ± 3.59	3.91 ± 0.75	<100 µg/mL	Conform
EL-B	2.86 ± 0.03	0.00		Conform
EN-C	3.55 ± 0.06	62.85 ± 0.89		Conform
EN-D	0.00	72.93 ± 0.92		Conform
EN-E	21.23 ± 0.32	3.82 ± 0.12		Conform
EN-F	0.00	43.71 ± 1.5		Conform

due to spectral overlap with other species in the matrix [13] [18]. Capillary electrophoresis (CE) analyses are fast and economical compared to conventional chromatography while being equally efficient. Thompson and Trenerry (1995) [19] proposed a rapid method for the determination of ten commonly used synthetic food dyes permitted in confectionery by CE. Therefore, the non-accessibility of the equipment by the majority of laboratories especially in Africa is a limitation to its use.

Moreover, a wide range of techniques based on high performance liquid chromatography (HPLC) have been used to analyse food dyes and most of them were coupled with UV-Visible or MS detectors. As food dyes are very polar molecules, they elute very quickly near the dead volume making their separation difficult. In this case, ion-pairing HPLC can be performed by adding ion-pairing agents (hydrophobic ionic) such as ammonium acetate buffer, 1-hexadecyl trimethylammonium bromide (CTAB) and so, to the mobile phase to improve the retention of ionic analytes [14] [24]. The LC-UV/Vis methods developed by Dossi *et al.* (2006) and Culzoni *et al.*, (2009) [20] [21] required a complex elution gradient program, a detection system with different wavelengths or complex logarithmic assistance, and too long analysis time. The HPLC assay method developed by Amin *et al.*, [14] is not suitable for a simultaneous assay of our two analytes because the sunset yellow was eluted 20 min later after tartrazine.

We, therefore, opted for ion-pairing HPLC using ammonium acetate as ion-pairing agent, which is much more accessible and less precipitable than tetrabutylammonium. Chromatographic conditions, especially the composition of mobile phase, were optimized through several assays to achieve good resolution and better peaks shapes for the analytes. In this study, it was found that the mixture of acetonitrile and 10 mmol/mL ammonium acetate as mobile phase could achieve this purpose, and finally adopted because it produced good sensitivity and reproducibility. The elution gradient developed allows a good separation of our analytes with better analysis run time. The sample processing method was also quite simple.

Our results showed that the concentrations of soft drink samples in tartrazine and sunset yellow were all below the upper limit value of 100 µg/mL recommended by Codex Alimentarius and FDA. Our samples therefore were conforming [13] [25].

Our findings are different from those of Lawal *et al.*, (2020) in Nigeria on five brands of soft drinks which revealed that 40% of these drinks had concentrations higher than 200 µg/mL [13]. These high concentrations could come from the non-specificity of the spectrophotometer UV-Visible measurements due to spectral overlap of several chemical species in the sample. Our results are also different from those of Amin *et al.*, (2014) on the concentration of tartrazine in artisanal yoghurt in Abidjan, Côte d'Ivoire, which revealed that 4.8% of samples exceeded the 300 mg/kg [14]. This overdose can be explained by the artisanal nature of the manufacturing process, which implies a poor control or a non-existence

of good manufacturing practices.

Finally, it is important to notice that there was a variation in the concentration of tartrazine and sunset yellow in samples from different batches within the samples of AL-A brand. This could mean that the good manufacturing practices were not well respected by that manufacturer.

5. Conclusion

A simple, rapid, accurate and robust HPLC/UV-Visible method was developed and validated for simultaneous identification and quantitation of tartrazine and sunset yellow. This method was successfully applied to determine tartrazine and sunset yellow in soft drinks sold in Benin. From the obtained results of all the validation parameters, we can conclude that the developed method could be successfully applied for routine quality control of soft drinks with adequate precision and accuracy.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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