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Determination of Food Colorings in Pharmaceutical Preparations and Food Additives by a Validated HPLC Method

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Abstract— Synthetic azo dyestuffs are preferred in many products today because synthetic dyes are more resistant to environmental factors and their production is relatively easy and cheap. Despite their commercial advantages, they have very important toxicological risks to its consumers. Tartrazine (E102), one of the most commonly used yellow food dye, is a synthetic lemon yellow azo dye used as a food coloring. Sunset yellow (E110) a petroleum-derived orange azo dye, also known as yellow dye #6, is used to give foods an orange-yellow color. Both food dyes have serious toxicological risks, that are mutagenic and carcinogenic. For this reason, it is very important that the measurement of quantities in food products and pharmaceutical preparations by a fast, reliable and sensitive method. The aim of this study, developed and also validated a new high-performance liquid chromatography (HPLC) method equipped with diode array detector (DAD) which was simultaneous determination for tartrazine and sunset yellow in pharmaceutical preparations and food supplements.

The chromatographic separation was carried out a reverse-phase C18 analytical column, 4.6 mm x 250 mm, 5 µm particle size, at 40°C. The mobile phase prepared as a mixture of pH 4 oxalate buffer, methanol, water (7:2:1, v/v) was isocratically applied to the column at 1 mL/min flow and, diode array detector was set at 432 nm and 480 nm for the determination of tartrazine and sunset yellow, respectively. The samples were loaded into the HPLC as a 20 µL.

Total analysis time for simultaneous determination was below 6 min. The linear range for tartrazine and sunset yellow were between 0.2 and 20 µg/mL. Quantification limits of tartrazine and sunset yellow were 0.07 µg/mL and 0.19 µg/mL, respectively. Precision of the method for tartrazine sunset yellow were 4.0% and 7.1%, respectively. Recovery values in pharmaceutical samples which applied at 1, 2 and 5 µg/mL were found to be range from 87.7% to 104.3%. The proposed method shows excellent sensitivity, selectivity, and precision and has been satisfactorily applied for the determination of tartrazine and sunset yellow in a variety of total 12 real pharmaceutical preparations the and food supplements. This method applicable for routine food dyes monitoring especially in food analysis or in toxicology reference laboratories.

Index Terms— Tartrazine, Sunset yellow, HPLC, DAD, Validation, Pharmaceutical preparation, Food additives

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1 INTRODUCTION

Food dyes are usually added to various commercial food and beverage products in order to make them appear more attractive and to achieve the desired colour and to enhance the visual appeal of products. For safety reasons, there have been recent reductions in the number of permitted food colours but they are still being used all over the world because of their low price, effectiveness and high stability. In addition to this, the food processing industry uses all types of food colours, but to minimise potential toxicity the amounts of permitted synthetic colours, used are strictly limited [1,2].

Dyes divided into four categories; natural, nature-identical, inorganic and synthetic [3]. Natural and synthetic dyes are widely used in food and beverage manufacturing to enhance the visual appeal of food products. Synthetic dyes compared to natural dyes are more commonly used due to their relatively lower production cost and higher stability toward pH, colour infirmity, low microbial contamination, light and oxygen changes [4,5]. Sunset yellow and tartrazine are two synthetic yellow azo dyes commonly used in pharmaceuticals, food and beverages [6]. However, both may cause allergic and intolerance reactions, particularly among asthmatics and people with an aspirin intolerance. Other reactions can include migraine, blurred vision, itching, rhinitis and purple skin patche [7]. The concentration limits are 100 ppm and 50 ppm for tartrazine and sunset yellow, respectively, when used individually and no more than 100 ppm when used in combination, in accordance with EU Legislation [8]. Therefore, it is very important to develop appropriate, sensitive, and reliable methods for the determination of sunset yellow and tartrazine in pharmaceutical and food products.

Various methods have been developed for the determination of sunset yellow and tartrazine, such as enzyme-linked immunosorbent assay [9], differential pulse polarography [10], spectrophotometry [11], spectroscopy [12], thin layer hromatography (TLC) [13] and high-performance liquid chromatography (HPLC) [5,8,14]. In addition, low analytical sensitivity, inadequate intraday and inter-day reproducibility values and inappropriate recovery amounts cause problems for the use of these techniques. Although spectrophotometry is cheap and simple since interference from alkali and earth alkali group elements,

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the determination at low concentration levels is impossible [15]. Since, HPLC-UV assays are reliable, inexpensive and widely utilized; it has appeared to fit best for performing simultaneous separation, quantification and monitoring of tartrazine and sunset yellow as a primary concern of this paper.

A new developed and validated fast chromatographic method was developed and employed in the analysis of dyes in selected number of 12 pharmaceutic and foodstuff products. The method required a minimal sample preparation step that consisted of filtering the samples following dissolution by ultrasonic bath at 30 min in water:MeOH mixture.

2.3. Standard solutions

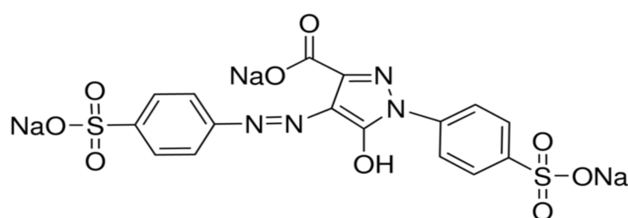
2 EXPERIMENTAL

2.1. Chemicals and reagents

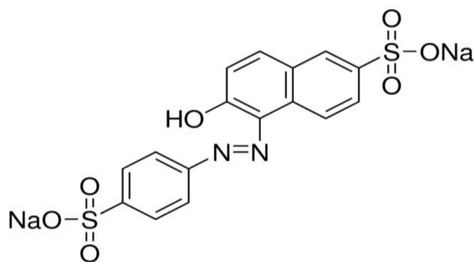
Pure reference samples of tartrazine (Figure 1.a) and sunset yellow (Figure 1.b.) (their purity ≥90%) were obtained from Alfa Easer and Aldrich, respectively. HPLC grade methanol obtained from Alfa Easer (Massachusetts, ABD). Analytical grade sodium hydroxide and sodium oxalate purchased from Merck (Darmstadt, Germany). 47 mm diameter membrane filter pore size were 0.45-µm used as filtration of mobile phase. Ultrapure water was produced by Elga Purelab system (High Wycombe, UK).

2.2. Instrumentation

The separation and quantification were performed by Shimadzu (Kyoto, Japan) high-performance liquid chromatography (HPLC) system which consist of a degasser, a gradient pump, an automatic liquid sampler with 20 µL sample loop, a column oven and a diode array detector (DAD). Analytical separation was performed by a C_{18} (250 x 4.6 mm) 5 µm particle size analytical column at 30 °C.



(a)



(b)

Fig 1. Chemical structure of tartrazine (a) and sunset yellow (b)

The mobile phase prepared as a mixture of pH 4 oxalate buffer, methanol, water (7:2:1, v/v) was used as a mobile phase. Mobile phase pH adjusted to 4.0 with 10 mM sodium hydroxide, filtered through a 0.45-µL filter and then, it was degassed for 30 min in an ultrasonic bath. Flow rate was 1.0 mL/min. The analytical separation was performed isocratically below 6 min. Tartrazine and sunset yellow were simultaneous quantified at 432 nm and 480 nm, respectively. The unknown concentrations of tartrazine and sunset yellow were quantified using linear regression of peak area response versus to analytes concentrations. System control and integrated data were recorded using the Empower TM computer software.

2.3. Standard solutions

Stock and working solutions of tartrazine (0.1 mg/mL) and sunset yellow (0.1 mg/mL) were prepared with methanol-water solution (7:3, v/v) and stored at -20°C until use. The working solutions of tartrazine and sunset yellow (0.2, 0.5, 1, 2, 4, 8, 12 and 20 µg/mL) were prepared weekly from the main stock solutions.

Likewise, quality control (QC) standards spiked with 1, 2 and 5 µg/mL of both tartrazine and sunset yellow were prepared to measure the repeatability values of the method. Also same protocol was used in preparation of limit of detection (LOD), limit of quantitation (LOQ) and recovery test samples.

2.4. Sample collection and its preparation to analysis

12 different samples were pharmaceuticals and food supplements were purchased from the pharmacy and herbal market. 5 tablets taken from each product were pulverized in a mortar and powdered 50 mg samples were solved with 5 mL MeOH/water (7/3, v/v) mixture in clean test tubes. The tubes were kept in an ultrasonic bath for 30 min at 25 °C for complete dissolution. Following, tubes were centrifuged at 4000 for 5 min. Finally, the centrifuged samples were filtered through 0.45 µm filter. 1 mL final products separated into the HPLC vials and they were injected to the HPLC as 20 µL by an automatic liquid sampler system.

2.5. Method validation

The developed analytical method was validated to demonstrate the specificity and selectivity, linearity, accuracy, precision, sensitivity and recovery. Intraday and inter-day validation protocol was applied considering reproducibility of the method to obtain accurate and precise measurements in agreement with Conference on Harmonization guidelines [16].

2.5.1. Specificity and selectivity

The method showed excellent chromatographic specificity without any interference at the retention times of tartrazine and sunset yellow (2.6 and 4.9 min). Representative chromatograms of spiked (Figure 2) samples illustrate the high resolution in below 6 minutes as the short separation time. The diode array detector of liquid chromatograph was set to 432 and 480 nm displaying for optimum sensitivity, for tartrazine and sunset yellow, respectively.

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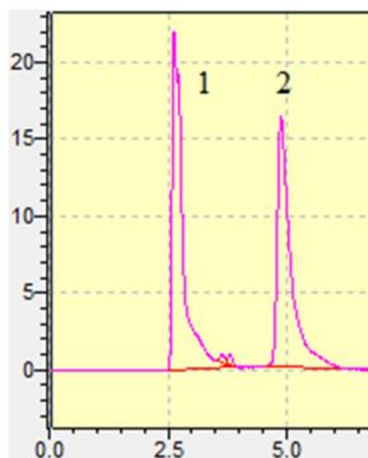

Fig.2. The chromatogram of example that contained tartrazine and sunset yellow as 10 mg/L concentration which is prepared by standard addition method used as quality control sample.

2.5.2. Linearity
After chromatographic conditions were established, calibration curves for tartrazine and sunset yellow were plotted concentrations over the range 0.2 - 20 µg/mL versus peak-areas to the concentrations. The calibration points, which were 0.2, 0.5, 1, 2, 4, 8, 12 and 20 µg/mL, were prepared by standard addition method.

2.5.3. Precision
The precision, defined as relative standard deviation (RSD), was calculated by separate replicates of tartrazine and sunset yellow both intraday and inter-day. Five replicate spiked samples were assayed intraday and inter-day at the three different concentrations 2, 5 and 10 µg/mL as representative of low, medium and high concentration for both analytes.

2.5.4. Sensitivity
The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the ICH guidelines [16] based on standard deviation of the response and the slope of the calibration graph.
LOD= 3.3σ/S; LOQ= 10σ/S (σ: The standard deviation of the response; S: The slope of the calibration curve). The concentration of 0.02 µg/mL as the lowest calibration points were used in sensitivity tests of methods.

2.5.5. Recovery
Extraction recoveries were determined by comparison of extracted samples of tartrazine and sunset yellow, to those from unextracted and directly injected standards, spiked with same amounts. Five individual replicates of spiked samples were prepared at two concentrations (1 and 2.5 µg/mL for tartrazine; 2 and 5 µg/mL for sunset yellow). Extraction procedure was carried out as described in the section of 2.4.

3 RESULTS AND DISCUSSION

3.1. Linearity
Calibration curves of tartrazine and sunset yellow drawn at 8 points between 0.2 - 20 µg/mL. Its concentration was prepared by the standard addition method and the obtained excellent correlation coefficiencieny with r^2 = 0,9986 and 0,9996, respectively (Figure 3).

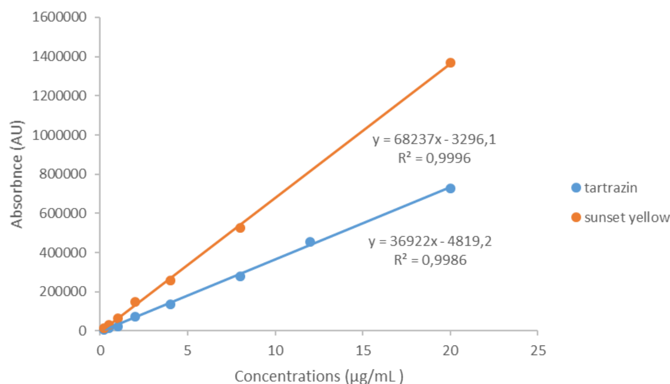

Fig.3. The calibration graphs for tartrazine and sunset yellow prepared in plasma by standard addition method

3.2. Precision
The data obtained from the precision tests (Table 1) performed in intraday and inter-day with quality control standards established in pharmaceutical samples which known to not have any food dyes by standard addition method showed low RSD% ≤8.20 values.

3.3. Sensitivity
The results of LOD and LOQ values, which were obtained by the measurement of individual 10 quality control (QC) samples, (Table 2) demonstrated that developed method has very low sensitivity values.

3.4. Recovery
Peak area ratios were compared and recoveries were calculated between 87.7% and 104.3% for each analyte tabulated in Table 3. The recovery results of the applied extraction procedure showed that the procedure has very high efficiency.

3.5. Results of tartrazine and sunset yellow in pharmaceutical and food additive samples
Tartrazine and sunset yellow levels in total of 12 pharmaceutical and food additive samples were monitored by developed and validated HPLC method. Analyzes were performed by 50 mg samples. None of these samples showed any problem for the quantification of the analytes, additionally, peak purity showed that no analytical interference was encountered from substances. The obtained results are given in Table-4.

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Table 1. Confidence parameters of validated method; intraday and inter-day precision and accuracy for determination of tartrazine and sunset yellow.

Conc. (ng/mL)	Intraday			Inter-day		
	No. Obs.	Estimated conc. $\bar{x}$±SD (µg/mL)	Precision (RSD%)	*No. Obs.*	Estimated conc. $\bar{x}$±SD (µg/mL)	Precision (RSD%)
2	5	2.35 ± 0.03	1.15	3	2.35 ± 0.12	4.9
5	5	4.91 ± 0.24	4.89	3	4.81 ± 0.09	1.77
10	5	10.51 ± 0.57	5.38	3	10.08 ± 0.38	3.77
2	5	2.03 ± 0.06	2.72	3	2.08 ± 0.17	8.20
5	5	4.87 ± 0.24	5.02	3	4.79 ± 0.12	2.54
10	5	10.11 ± 0.13	1.33	3	10.06 ± 0.05	0.45

Table 2. Limit of detection and quantification results of the method

Dye	LOD	LOQ	RSD%
Tartrazin	0.02	0.07	4.04
Sunset Yellow	0.06	0.19	7.08

Table 3. Recovery results of tartrazine and sunset yellow

Dye	Conc. (µg/mL)	Recovery%	RE%
Tartrazine	2	90.33	-9.67
	5	87.73	-12.27
Sunset Yellow	1	104.33	4.33
	2.5	103.33	8.33

Table 4. Pharmaceuticals and food additives samples analysis results

Sample number	Tartrazine (mg/g)	Sunset Yellow (mg/g)
1	ND	0.637+0.0152
2	0.73+0.02	ND
3	ND	0.113+0.0111
4	ND	0.5835+0.0035
5	0.0168+0.0004	ND
6	ND	0.028+0.001
7	0.5393+0.0154	ND
8	ND	0.0315+0.0017
9	0.1103+0.0174	ND
10	0.061+0.0017	ND
11	0.2060+0.0038	0.0906+0.0051
12	0.4613+0.0217	ND

4 CONCLUTIONS

The HPLC method developed in this article is rapid, specific, and sensitive. The precision test result of the method, which are RSD% ≤8.20 that is very good result [16] therefore reliability of the developed method is very high. The obtained excellent recovery values between 94.4% and 106.6% with (-12.3) and 4.3 RE% values would be attracting the use of this method. Because of the simplicity of sample preparation, short analysis time (<6 min) and the high sensitivity of presented technique make particularly attractive for the quantification of tartrazine and sunset yellow in pharmaceutical and food additives samples. We strongly recommended this validated method to be used in routine analysis of tartrazine and sunset yellow. In this work it is revealed that established HPLC-DAD method is suitable for the routine monitoring of tartrazine and sunset yellow in reference food analysis laboratory and it applicable as a reference method in routine monitoring these dyes for toxicological and/or analytic purposes. The proposed method can be easily applied to many kinds of foods and beverages samples which are important to the toxicologically.

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CONFLICT OF INTEREST STATEMENT

None of the authors of this article has a financial or personal relationship with other persons or organizations that may inappropriately affect or bias the content of the paper. The authors declare that there is no conflict of interests.

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