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Research Article

RAPID DETERMINATION OF TARTRAZINE IN SELECTED FOODSTUFFS COMMONLY CONSUMED IN OUJDA CITY (MOROCCO) BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV-DAD DETECTION

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ABSTRACT

The present study deals determination of Tartrazine, which is known as an azo dye, present in three different kinds of yellow foodstuffs: solid juice powders, solid jelly powders and soft drinks commercially available food items in Oujda- Morocco markets.
Tartrazine present in different kinds of yellow foodstuffs was determined by high-performance liquid chromatography with UV-DAD detection. The following food dye was analyzed within less than 10 min using analytical C18 column (4.6 × 150 mm, 10 µm) at ambient temperature.
This method has potential to be used for Tartrazine due to its rapidness, simplicity, reliability, and sensitivity.
All studied samples showed dye levels in according to the legislations adopted by the official food control laboratories in Morocco.

Keywords: Synthetic food colours; Tartrazine; HPLC; UV –DAD detector; Morocco.

INTRODUCTION

Food additives are used in processed foodstuffs to restore the natural colours lost in processing, to reduce the batch-to-batch variations inherent with natural colours, and to produce products that are aesthetically and psychologically attractive. When compared to natural dyes, synthetic dyes show several advantages such as high stability to light, oxygen and pH, colour uniformity, low microbiological contamination, relatively lower production costs, etc. (Hathcock & Rader, 2003).

The use of food dyes is at least controversial because they are only of esthetical role. Moreover many of them have been related to health problems mainly in children that are considered a very vulnerable group (Clydesdale, 1993; Polônio, 2002). Furthermore in some cases the use of food dyes is also indicative of foodstuff adulteration such as in their addition to fruit juices (Kiseleva, Pimenova, & Eller, 2003). Generally, synthetic dyes contain azo (N=N) functional groups and aromatic ring structures, so they are harmful to human health (López-de-Alba et al., 2001).

Among the food dyes which are widely used is Tartrazine. It is an orange-coloured, water soluble powder used worldwide as food additives to colour several foods, drugs and cosmetics. It has the following chemical structure illustrated in Fig.1.

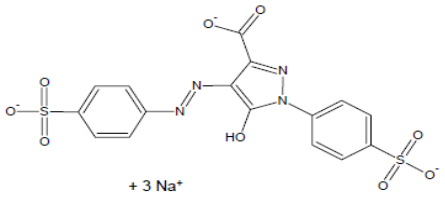

Fig. 1: Chemical structure of tartrazine (trisodium salt of 3-carboxy-5-hydroxy- 1-(p-sulphophenyl)-4-(p sulphophenylazo) pyrazole.

Moreover, this food colorant is used in cooking in many developing countries as a substitute for saffron (Mehedi, et al., 2009) The Acceptable Daily Intake (ADI) for humans is 0-7.5 mg kg^{-1} body weight (JECFA, 1965).

The European Union (Directive 94/36//EC, 1994) has issued comprehensive schemes that regulating the use of food colours and their allowed levels in all food products. Such regulations will guide the analytical chemists for monitoring synthetic dyes in food products. According to the legislations of the European Union for food dyes (which is the same legislations adopted by the official food control laboratories in Morocco) table 1.

Table 1: Maximum allowable concentrations in the studied foodstuffs according to the Moroccan legislation.

	Concentrate (liquid or solid) fruit juice, fruit nectar (mg/100g)[b]	Confectionery (hard and soft candy) (mg/100g) [a]	Soft drinks (mg/100ml) [a]
Tartrazine	30	30	10
the number of samples	7	24	10

[a] **(JOINT FAO/WHO, December 2010).**
[b] **(Directive 94/36//EC, 1994).**

The aim of this study was to determine the quantitative contents of Tartrazine in three different kinds of yellow foodstuffs: solid juice powders, solid jelly powders and soft drinks, and compare the intake levels to ADIs set by the FAO/WHO.

MATERIALS AND METHODS

Chemicals

Tartrazine (CAS 1934-21-0, Purity 86.7%) from Alfa Aesar (Germany) was employed. All reagents: Methanol, ammonium acetate, EDTA, sodium acetate and acetic acid were of analytical grade and were purchased from Sigma Aldrich. Double distilled water was distilled with WD Series Automatic Water Still.

Preparation of Standard Stock Solution

Standard stock solution of Tartrazine containing 100 mg/L was prepared by weighing sufficient amount of the correspondent solids followed by dilution to 100 ml with double distilled water water. Working standards of individual dyes were prepared by dilution of aliquots of the stock

solutions (Simone Pereira Alves et al, 2008). A representative chromatogram of standard dye is shown in Fig.2.

Linearity and calibration standards

The linearity of the method was calculated using various concentrations of Tartrazine (1, 5, 10, 15 and 20 mg/l) in triplicate. Quantification of Tartrazine in samples was carried out on the basis of peak areas and comparison with a calibration curve obtained with the corresponding standards.

The calibration Curve was plotted taking peak area ratio on x-axis against concentration of Tartrazine on y-axis (Fig.3.).

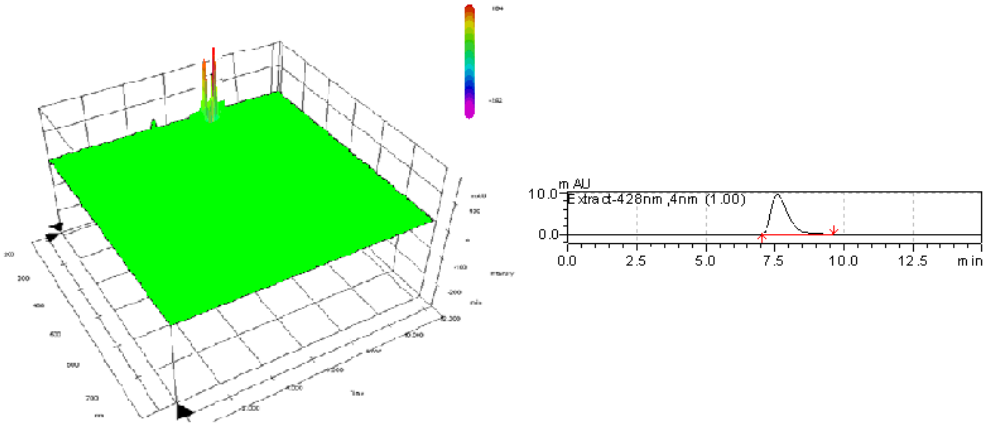

Fig.2: HPLC 428 nm spectrum and three-dimensional chromatogram of Tartrazine (pure dye) 10 mg. ml^{-1} (max at 8.306min).

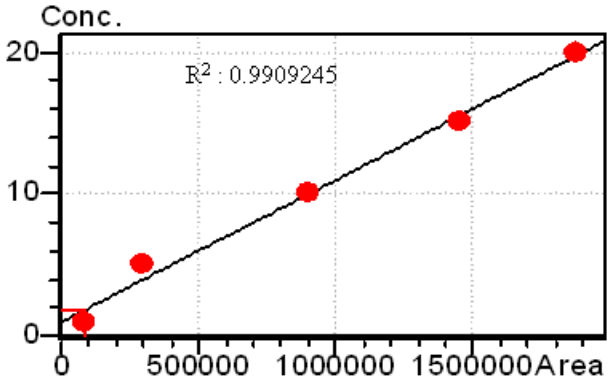

Fig.3: Calibration curve for Tartrazine (The coefficient of determination (R^2) obtained was 0.9909245).

Preparation of Sample Solution

Samples consisted of solid Concentrate (liquid or solid) fruit juice, fruit nectar, Confectionery (hard and soft candy) and soft drinks. Samples were of commercial products usually sold in the local market. They were bought in the markets of Oujda city, Morocco.

Solid samples were previously homogenized in their own packages before sampling. 3 g of Samples were precisely weighted. Concentrate solid fruit juice samples were directly dissolved in Double distilled water at room temperature. Confectionery (hard and soft candy) samples were dissolved in hot Double distilled water (~60 °C). In both cases they were diluted up to 50 ml with Double distilled water and filtered through 0.45 µm filters. Soft drink samples were previously degassed in ultrasonic bath, filtered through 0.45 µm filters and directly analyzed.

Instrumentation and chromatographic conditions

The chromatographic system consisted of Shimadzu model LC-6AD, CBM 20A controller and SPD-M20A multi-array detector (DAD: diode array detector). HPLC analysis was conducted using C18 reversed-phase column (ODS C18: 250x 5mm, 5µm). The mobile phase consisted of methanol (solution A) and aqueous ammonium acetate (0.08 mol/L; solution B). Prior to use the aqueous solutions and methanol were degassed in ultrasonic bath. Aqueous solutions were further filtered through 0.45 µm membranes. Elution was carried out at 0.4 ml/min. Isocratic conditions were employed for both solvent systems and the mobile phase was composed of 45% of solution A and 55% of solution B. The injection volume is 20 µl. The effluent is monitored for UV absorption at 428 nm. All separations are performed at ambient temperature (Simone Pereira Alves et al, 2008).

Tartrazine identification and quantification

Optimum absorption wavelength of Tartrazine was evaluated using standard solutions. Tartrazine was identified by comparison with retention time of standard solution and by their absorption spectra. The method allowed the detection of the Tartrazine in less than 10 min leading to a high throughput of sample evaluation.

Statistical analysis

Data are presented in tables as the mean ± SEM. The statistical significance of the differences between control and experimental groups was evaluated by Student's *t*-test using GraphPad Instat 3.06 (Fisher, R.A., 1950).

RESULTS & DISCUSSION

Tables 2-4 presents the concentrations (mg/100 g) of Tartrazine identified in a variety of the foodstuffs that are most commonly consumed in Oujda (Morocco). As expected, most of the studied foodstuffs showed two or three dyes in their composition due to the desired colours of final products.

Among the 41 food items analysed, 28 samples, i.e., about 68.3 %, contained Tartrazine, thirteen food items out of 41 analysed, i.e., about 31.7% of the total items analysed, did not contain Tartrazine.

The concentrate (liquid or solid) fruit juice, fruit nectar; confectionery (hard and soft candy) and soft drinks contain Tartrazine respectively: 100 %, 58.33 % and 70%.

Tartrazine concentrations varied widely between 0.22 and 23.45 mg/100 g in the studied products depending on the considered flavour, brand and

foodstuff. It is worth of note that Tartrazine concentrations were always below the maximum legislated values.

Table 2: Tartrazine concentrations (mg/100g) (mean ± standard deviation) obtained for different samples of Concentrate (liquid or solid) fruit juice, fruit nectar.

	Samples	Concentrations (mg/100g)
Concentrate (liquid or solid) fruit juice, fruit nectar	1	1.45 ± 0.02
	2	0.98 ± 0.01
	3	4.30 ± 0.03
	4	1.11 ± 0.02
	5	23.45 ± 0.04
	6	6.09 ± 0.05
	7	16.51 ± 0.02

[a]All values are presented as the mean ± SD of triplicate determinations.

Table 3: Tartrazine concentrations (mg/100g) (mean ± standard deviation) obtained for different samples of Confectionery (hard and soft candy).

	Samples	Concentrations (mg/100g) [a]
Confectionery (hard and soft candy)	1	ND [b]
	2	0.57 ± 0.02
	3	1.06 ± 0.01
	4	ND
	5	0.24 ± 0.01
	6	ND
	7	ND
	8	2.31 ± 0.04
	9	0.49 ± 0.03
	10	2.44 ± 0.02
	11	0.24 ± 0.05
	12	ND
	13	0.89 ± 0.06
	14	ND
	15	ND
	16	ND
	17	1.23 ± 0.02
	18	0.22 ± 0.01
	19	0.50 ± 0.05
	20	1.32 ± 0.04
	21	0.77 ± 0.05
	22	ND
	23	0.35 ± 0.03
	24	ND

[b] All values are presented as the mean ± SD of triplicate determinations.
[a] ND = not detected.

Table 4: Tartrazine concentrations (mg/100g) (mean ± standard deviation) obtained for Soft drinks.

	Samples	Concentrations (mg/100g) [a]
Soft drinks	1	1.85 ± 0.02
	2	0.35 ± 0.03
	3	0.73 ± 0.04
	4	ND [b]
	5	1.22 ± 0.04
	6	1.33 ± 0.05
	7	ND
	8	0.73 ± 0.01
	9	ND
	10	0.23 ± 0.02

[a] All values are presented as the mean ± SD of triplicate determinations.[b] ND = not detected.

Nevertheless our results show the importance of displaying dye concentrations in the labels of commercial products since that data would allow the final consumer to choose between brands, flavours and products selecting those containing the lowest concentrations quantities and number of dyes.

This possibility of choice is more pertinent in products containing Tartrazine that is suspected of causing several health problems (Beseler, 1999; Ortolani et al., 1999). Furthermore it has to be considered that most of the studied foodstuffs have children as their major final consumers and this way their higher physiological susceptibility in neglected (Simone Pereira Alves and al, 2008).

CONCLUSION

From the data obtained, it has been shown that Tartrazine is widely used in several foodstuffs that are commonly consumed in Oujda (Morocco), and that his level is according to the legislations adopted by the official food control laboratories in Morocco.

It can be concluded that the presented method has potential to be used for Tartrazine due to its rapidness, simplicity, reliability, and sensitivity.

The validated method represents an alternative to methods of analysis of several dyes including those that usually do not occur simultaneously in real samples. It can be useful in quality control and it shows also a high throughput due to the use isocratic conditions that reduce the stabilization time necessary between consecutive determinations.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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