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Research Article

Simultaneous estimation of colorants Sunset Yellow and Tartrazine in food products by RP-HPLC

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ABSTRACT

An accurate, rapid and sensitive RP-HPLC method was developed and validated to simultaneously estimate sunset yellow and tartrazine in food products. Analysis was performed using waters 1515 isocratic HPLC system (pump); A Waters 2487 dual wavelength detector was used for analysis with a Waters Breeze version 3.3 data station for data collection and processing. Separation was achieved using a Phenomenex C18 column, (250 × 4.6mm, 5µ), A mixture of 50 mM potassium dihydrogen orthophosphate buffer (adjusted to pH 7.5): acetonitrile (80: 20v/v) was pumped at 0.7 mL/min for separation. The eluents were monitored at 244 nm. A solution containing 0.5gm of both Sunset yellow and Tartrazine were prepared from a commercially available food product and then used for evaluation. 15µg/ml solution of riboflavin was used as internal standard. The developed method was validated as per ICH guidelines. The separation with the above said conditions gave a sharp and symmetric peak. Linearity for Tartrazine and Sunset yellow were observed in the concentration range of 0.5 – 3.0 µg/mL for both with a correlation of 0.999. The recoveries were found to be 85.5 and 83.3 % respectively. In conclusion a simple, sensitive and rapid RP HPLC method was developed for simultaneous estimation of Tartrazine and Sunset yellow in food products.

Keywords: Sunset yellow; Tartrazine; RP-HPLC; Validation; Food analysis.

1. INTRODUCTION

Sunset yellow is chemically designated as 6 – hydroxy – 5– [(4-sulfophenyl) azo] – 2 napthalenesulfonic acid disodium salt, 1-p- sulfophenylazo – 2 – naphthol – 6 – sulfonic acid disodium salt, Tartrazine is chemically designated 4,5-dihydro–5–oxo–(4-sulfophenyl)4-[(4-sulfophenyl)azo]-1*H* –pyrazole—3-caboxylic acid trisodium salt (Arthur H Kibbe, 3rd edn; The Merck Index, 13th edn). They are basically water soluble dyes used as coloring agents in food products. A colour additive is any dye, pigment or substance which when added or applied to a food is capable of imparting colour. The colour additives added should be regulated, to ensure that foods containing colour additives are safe to eat, contain only approved ingredients and are accurately labeled. Colour provides visual information about a food's quality, condition. One of the most important reasons for analyzing foods from the consumers and manufacturers stand point is to ensure that they are safe. The food manufacturers should continuously try to increase their market share and profits. To do this they must ensure that their products are of higher quality, less expensive and more desirable. To meet these vigorous standards food manufacturers need analytical techniques to analyze food materials before, during and after the manufacturing process to ensure that the final product meets the desired standards. With reference to the "Prevention of food adulteration act, India (2004)", the maximum limit of permitted synthetic food colours or mixture there if which may be added to any of food article shall not exceed 100 parts per million of the final food or beverage for consumption, except in case of food articles like peas, strawberries and cherries in hermetically sealed containers, preserved or processed papaya, canned tomato juice, fruit syrup, fruit squash, fruit cordial, jellies, jam, marmalade, candied crystallized or glazed fruits where the maximum limit of permitted synthetic food colours shall not exceed 200 parts per million of the final food or beverage for consumption.

Literature survey reveals that there are several Spectrophotometric (Berzas Nevado JJ et al, 1995; Capitan

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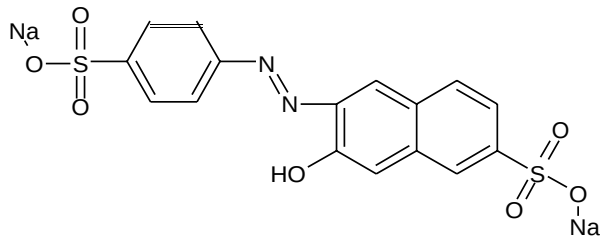
Vallvey LF et al, 1997; Derya Kara, 2005; Mahmure ustan ozgur and Ikbal koyuncu, 2002; Erdal Dinc, et al, 2002), HPLC (Gonzalez M et al, 2003; Erdal Dinc et al, 2006; Szulinska Z et al, 2005; Katerina S Minioti et al, 2007), LC-MS (Ma M, Luox et al, 2006) and electrophoresis (Huang HY et al, 2002; Oishi M et al, 2004) methods available for the analysis of sunset yellow and tartrazine in individual forms and in combination with other approved colours. However, there are no methods reported for simultaneous estimation of tartrazine and sunset yellow in combined form. Many of the food products producing orange shade were found to contain a mixture of tartrazine and sunset yellow. The purpose of the present study was to evaluate sunset yellow, tartrazine in food products by an isocratic RP-HPLC method with UV detection. The developed method was validated as per ICH guidelines (Validation of Analytical Procedures, 1996). The chemical structures of sunset yellow and tartrazine are shown in Fig. 1.

Sunset yellow



Tartrazine

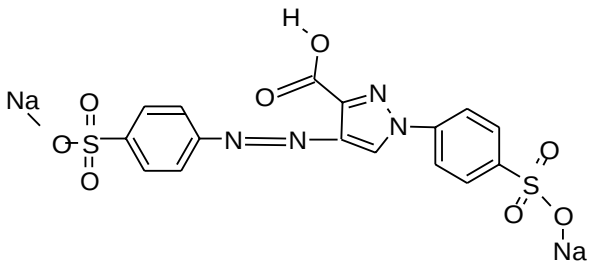


Figure 1: Structures of sunset yellow and Tartrazine

2.0 MATERIALS AND METHODS

2.1. Chemicals and Reagents

HPLC-grade Acetonitrile, Methanol and analytical grade Potassium dihydrogen orthophosphate, Orthophosphoric acid, Triethylamine were purchased from SD Fine Chemicals Limited (INDIA). The water used was ultrapure water collected from Millipore system. Food colorants - Tartrazine and Sunset yellow were obtained as gift samples from strides arco labs (INDIA).

2.2. Instrumentation

The measurements were performed with a WATERS HPLC system - Waters dual wave length(2487) detector, Waters 1515 isocratic solvent delivery system (pump), Waters Breeze version 3.3 data station, Rheodyne – 7125 injector with a 50microlitre loop volume; Separation was achieved using a Phenomenex C18 column, (250 × 4.6mm, 5µ). During analysis Sartorious digital balance, Systronics pH meter and Ultrasonicator were also used.

2.3. Chromatographic conditions

Seperation was achieved using a Phenomenex C_{18} column, (250 × 4.6mm, 5µ). The solvent system consisted of 50mM potassium dihydrogen orthophosphate buffer (Adjusted to pH 7.5 using orthophosphoric acid): acetonitrile (80: 20v/v) was pumped Isocratically at 0.7 mL/min for separation. The eluents were monitored at 244 nm.

2.4. Preparation of Stock solutions

1mg/ml solution of both sunset yellow and tartrazine were prepared individually by dissolving in distilled water. From above 100µg/ml solutions were prepared individually. 1mg/ml of riboflavin (Internal Standard) solution was prepared. From the above solution working standards ranging from 0.5µg/ml, 1.0µg/ml, 1.5µg/ml, 2.0µg/ml, 2.5µg/ml and 3.0µg/ml of each sunset yellow and tartrazine were prepared containing 15µg/ml of internal standard. The standard chromatogram is presented in Fig. 2.

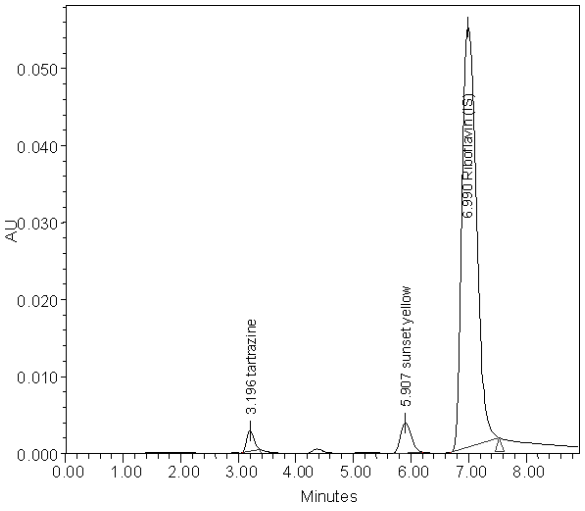


Figure 2: Typical chromatogram of a standard solution of tartrazine and sunset yellow (0.5 µg/mL each)

2.5. Sample preparation

0.5009gm of custard powder was accurately weighed and transfered in 10ml volumetric flask. To this 1.5ml of acetonitrile was added and sonicated for 15min.

Then the volume was made up to 10ml with acetonitrile and filtered using whatmann filter paper. To 1ml of the filtrate, 1.5ml of 100µg/ml solution of internal standard was added and diluted to 20ml with mobile phase.

This was injected in to the system and the sam- ple chromatogram was recorded and presented in Fig. 3.

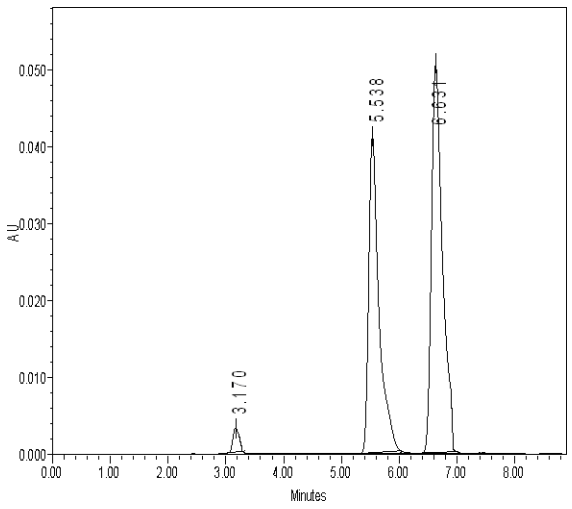


Figure 3: Typical chromatogram of a sample solution (Custard powder)

2.6. Calibration curve solutions used for method validation

Standard solutions of 0.5 to 3.0µg/ml of sunset yellow and 0.5 to 3.0µg/ml of tartrazine were prepared by diluting the stock solution with the mobile phase to reach concentrations and were analyzed to check the linearity of response (Table. 1).

triplicate analysis of each calibration solutions. Linear correlations were obtained over the range studied, with correlation coefficients ≥ 0.999 for standards. The regression equation was y = 0.0912x + 0.003 (R2=0.999) for sunset yellow and y = 0.0474x - 0.0015 (R2=0.999) for tartrazine.

3.3. Accuracy

The accuracy of the method was determined by recovery experiments. The recovery of the method was determined at single level by adding a known quantity of sunset yellow and tartrazine to the food products of pre analyzed samples and the mixtures were analyzed according to the proposed method. The percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table. 2.

3.4. Precision

The precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated and presented in Table. 3. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of peaks and percentage RSD were calculated and presented in Table. 3.

Table 1: Linearity and range

S.No	Concentration of tartrazine (µg/ml)	Concentration of sunset yellow (µg/ml)	Concentration of Riboflavin (µg/ml)	Response factor for Tartrazine	Response factor for sunset yellow
1	0.5000	0.5000	15.0000	0.021281	0.048174
2	1.0000	1.0000	15.0000	0.04614	0.094826
3	1.5000	1.5000	15.0000	0.070505	0.140861
4	2.0000	2.0000	15.0000	0.093992	0.183675
5	2.5000	2.5000	15.0000	0.1161	0.229349
6	3.0000	3.0000	15.0000	0.140555	0.277932

Table 2: Analysis of custard powder

S. No	Sample	Amount found* µg / gm		% Recovery*	
		E102 ± RSD	E110 ± RSD	E102 ± RSD	E110 ± RSD
1	Smith and Jones Custard powder	97.20 ± 0.048	1056.48 ± 0.047	85.56 ± 0.011	83.80 ± 0.014

3.0. VALIDATION

3.1. *Specificity*

The specificity of the method was ascertained by analyzing the standards and samples. The peaks of sunset yellow and tartrazine in sample were confirmed by comparing the retention time and spectra of the standards.

3.2. *Linearity and Range*

Linearity was assessed with the aid of serially diluted calibration solutions. The standards were injected separately. Calibration graphs were plotted on the basis of

3.5. Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ of the developed methods were determined by injecting progressively low concentration of the solid solutions using the developed RP-HPLC method. The limit of detection is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The limit of quantification is the smallest concentration of the analyte, which gives a response that can be accurately quantified (signal to noise ratio of 10) (Table. 4)

Table 3: Precision studies

Intraday studies				Interday studies				
RF* of Tartrazine	**Mean ± RSD***	**RF of Sunset yellow**	**Mean ± RSD**	**Day**	**RF of Tartrazine**	**Mean ± RSD**	**RF of Sunset yellow**	**Mean ± RSD**
0.0968 0.0989 0.1006 0.1040 0.1057 0.1001	0.1011 ± 0.0329	0.1930 0.1931 0.1930 0.1918 0.1931 0.1930	0.1929 ± 0.0028	Day I	0.0970 0.0986 0.1009 0.1026 0.1012 0.1045	0.1008 ± 0.0267	0.1932 0.1930 0.1919 0.1926 0.1934 0.1932	0.1929± 0.0029
				Day II	0.1006 0.1023 0.1045 0.1040 0.0993 0.1003	0.1018 ± 0.0207	0.1920 0.1924 0.1933 0.1933 0.1921 0.1930	0.1927± 0.0031
				Day III	0.0998 0.1002 0.1003 0.1032 0.1044 0.1026	0.1017 ± 0.0028	0.1939 0.1936 0.1925 0.1918	0.1930 ± 0.0039

3.6. Ruggedness and Robustness

The ruggedness of the proposed method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC-10AT), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil C_{18}, Phenomenex LUNA C_{18} and Hichrom C_{18}. Robustness of the method was determined by making slight changes in the chromatographic conditions as stated in ICH guidelines.

3.7. System suitability testing

System suitable studies were carried out as specified in the United States pharmacopoeia (USP). These parameters include column efficiency, resolution, peak asymmetry factor, capacity factor and percentage coefficient of variation of peak area or height on repetitive injections. Although the USP requires any two of this criteria for method validation, parameters like column efficiency (N), resolution (Rs) and peak asymmetry factor was calculated in the present study. Chromatograms of mixed standard solutions were used for these calculations.

Table 4: Validation and system suitability studies

S. NO	PARAMETERS	TARTRAZINE	SUNSET YELLOW
1	Linearity and range	0.500 to 3.0 µg/ml	0.500 to 3.0 µg/ml
2	Regression equation Y = mx + c*	y = 0.0474x - 0.0015	y = 0.0912x + 0.003
3	Correlation coefficient	R2 = 0.9997	R2 = 0.9998
4	Theoretical plates/meter	10214.416	8723.1
5	Resolution factor	4.17	
6	Asymmetric factor	1.05	1.00
7	Limit of detection (ng/ml)	3.0 ng/ml	3.0 ng/ml
8	Limit of quantitation (ng/ml)	9.0 ng/ml	9.0 ng/ml

4.0. RESULT AND DISCUSSIONS

Optimization of the method was carried out using various concentrations of acetonitrile while keeping the pH of the aqueous phase constant. A solvent combination of 50mM potassium dihydrogen orthophosphate buffer (adjusted to pH 7.5): acetonitrile (80: 20v/v) gave a satis-factory separation of the compounds of interest.

This optimized mobile phase separated sunset yellow at 5.9 min and tartrazine at 3.1 min respectively. Increase in the concentration of the organic phase in the solvent system resulted in faster elution but loss of resolution. On the other hand increasing the aqueous phase concentration caused peak broadening and increase in retention time.

The calibration curves of both sunset yellow and tartrazine were linear in the range of 0.5-3.0 µg/ml. Linear regression equation and correlation coefficient are shown in Table. 4. Detection limit of both sunset yellow and tartrazine was found to be 3 ng, where as quantification limits were found to be 9 ng for both sunset yellow and tartrazine. Recovery of both the drugs from the sample matrices was between 82.00 – 86.00%.

No marked changes in the chromatogram occurred on changing the instrument; operator; columns and chromatographic conditions indicated that the developed method was rugged and robust.

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and are presented in Table. 4. The values obtained demonstrated the suitability of the system for the analysis of sunset yellow and tartrazine in combined form in food products.

5.0 CONCLUSION

The result obtained showed that the above method provided adequate accuracy, precision, sensitivity, reproducibility for the analysis of sunset yellow and tartrazine in combined form in food products. Thus it may be concluded that the proposed method can be used for the routine analysis of food products containing tartrazine and sunset yellow without any interference from each other.

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