e-ISSN: 2248-9126
Vol 4 | Issue 1 | 2014 | 49-52. Print ISSN: 2248-9118

Indian Journal of Pharmaceutical Science & Research

www.ijpsrjournal.com

IDENTIFICATION AND DETERMINATION OF BINARY MIXTURES OF SYNTHETIC DYES WITH CR (III) COMPLEXATION IN FOOD STUFFS AND PHARMACEUTICAL SAMPLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Amandeep Kaur and *Usha Gupta

Department of Chemistry, Punjabi University, Patiala, India.

ABSTRACT

Binary mixtures of synthetic dyes (tartrazine and erythrosine) using Cr (III) as complexing agent commonly used as colorant in food stuffs and pharmaceutical sample, were investigated on Intertsil ODS-3V C-18 column by High performance liquid chromatography (HPLC). The compositions of mobile phase containing methanol-water in acetate buffer were optimized at the maximum wavelength at each synthetic dye with Cr (III). Relative standard deviation of retention time 1.833-14.438 for binary mixture of synthetic dye with Cr (III) complexation were studied respectively. The optimal methods were applied to identify and determine the synthetic dyes in food stuffs and pharmaceutical samples.

Keywords: Synthetic dyes, Cr (III), Food stuffs, Pharmaceutical samples, High performance liquid chromatography.

INTRODUCTION

Natural or synthetic colorants are often added to food stuffs or pharmaceutical samples in order to maintain the natural color during process or storage and to create the desired colored appearance. However, synthetic dyes such as low price and high stability. At present, synthetic dye is widely used to make food more attractive and appetizing. Due to its toxicity, especially when consumed in excess, synthetic dye is strictly controlled by laws, regulations and acceptable daily intake (ADI) values for food safety (Table I). The chromophore groups in synthetic dyes can be analyzed with several methods individually such as visible spectrophotometry [1], thin layer chromatography [2], High performance liquid chromatography [3-5], capillary electrophoresis [6-9] and ion chromatography [10]. Among the methods mentioned above, HPLC provided the highest sensitivity and separation of synthetic dyes with reagent were performed on a C-18 column.

Tartrazine food dye belongs to azo group and erythrosine food colorant belongs to xanthene group. foods such as confectionery, cotton candy, soft drinks (Mountain Dew), energy drinks, instant puddings contain tartrazine in varying proportions. Tartrazine [11] appears to cause the most allergic and intolerance reactions of all the azo dyes, particularly among asthmatics and those with an aspirin intolerance [12].A variety of immunologic responses have been attributed to tartrazine ingestion, including anxiety, migraines, clinical depression, blurred vision, itching, general weakness, heat waves, feeling of suffocation, purple skin patches, and sleep disturbance[13]. It is most popularly used as a food coloring agent and a host of other applications [14] such as printing inks, a dental plaque disclosing agent, a biological stain, cosmetics, cocktails, tinned fruits, biscuits, chocolate, and snack foods. It is highly toxic, causes various types of allergies, thyroid activities, carcinogenicity, anemia, DNA damage behavior, neurotoxicity and xenoestrogen nature in the humans and animals.

Therefore, the purpose of this study was to develop HPLC method on Intertsil ODS-3V C-18 column for the separation of complexation Cr (III) with binary mixture of synthetic colorants under high sensitivity. The

Corresponding Author:- **Usha Gupta** Email: guptausha57@yahoo.com

proposed method was applied to identify and determine the synthetic dyes in food and pharmaceutical samples.

MATERIALS AND METHODS

Apparatus

The HPLC (Water 2695) set up consisting of a P 2489 quaternary solvent delivery pump, C 18 column of 250 × 4.6 mm (i.d.) filled with C 18 material (5.0 µm) (Intertsil ODS-3V) and a UVD 170 detector capable of detecting at eight wavelengths. The signal obtained was processed by EMPOWER 2 software package and the chromatograms were monitored on an interfaced computer. The flow rate of the mobile phase methanol: water (70:30) was 1.0 ml/min and the column temperature was kept at 30^{o}C. Each analysis was performed in three replicates.

Reagents and Solutions

The standard synthetic dyes were tartrazine (E102) and erythrosine (E127) from Merck. Stock solutions of synthetic dyes were prepared at a concentration of 1000mg/l. All dyes were dissolved in Milli Q water. HPLC methanol, sodium hydroxide and acetic acid were from Merck and Chromium chloride was from Sigma Aldrich.

Preparation of dye complexes

Aliquots of solution containing tartrazine and erythrosine in range of 25-150 ng/ml were taken in a 5.0 ml measuring flask. 1.0 ml of sodium acetate/acetic acid buffer of pH 5.5 and 0.5 µg/ml of Cr (III) reagent were added. The final volume was adjusted to 5.0 ml with Milli Q water.

RESULTS AND DISCUSSION

Absorption Spectra for dye complexes

Absorption spectra of each standard synthetic dye complexes with Cr (III) dissolved in methanol water measured at pH 5.5 is shown in Figure1. The maximum absorbance of each dye with Cr (III) occurred in both UV and visible wavelength. Due to UV cut off of methanol and acetate buffer interfered the absorbance of dye in UV range, detection wavelengths for HPLC was selected from the maximum absorbance in visible range at 400.0 nm for tartrazine , erythrosine with Cr (III) complexes.

Separation Parameters: The amount of methanol in the mobile phase strongly affected retention time. Binary mixture of synthetic dye with Cr (III) complexing agent used in the study exhibited different hydrophobic interaction to stationary phase. Therefore, the dye complexes were eluted form C-18 column using different percentage of methanol and small molecules containing high polarity was initially eluted. Other factors of mobile phase were investigated as follow: First, the concentration of acetate buffer over the range 10-15mM provided similar retention time and peak height. Thus 10mM acetate buffer was selected. Second, the eluent pH an effect on peak height and peak area of synthetic dyes and pH 3.5-7.0 was studied. The optimal eluent pH was selected from the highest peak height observed. The best pH was obtained from dye complexes at pH 5.5.

The separation of Tartrazine-Cr (III) and Erythrosine-Cr (III) complexes was performed by using C 18 column. The composition of eluents phase was varied from 70 to 95% (v/v) of methanol with water to optimize the separation of dye complexes. The use of 70% methanol composition provided a fast separation with sufficient resolution. The flow rate was optimized by the variation of flow from 0.5 to 1.0 ml/min. A sufficient separation was observed at a flow rate of 1.0 ml/min but column pressure was high using a mobile phase of methanol: water (70:30). A chromatogram showing the separation of tartrazine, erythrosine complexes with Cr (III) (Figure 2).

Analytical performance parameters: The limit of detection (LOD) was calculated for the dye complexes. The accuracy (% recovery) and precision (% RSD) of HPLC-UV method were checked three times for each analyte by analyzing a standard solution of known concentration (40.0 ng/ml) and quantifying it using standard calibration curves. The results of analysis are given (Table II).

Application: The developed procedure has been applied to fruit syrup (Kissan Mango), candy (Mango Bite), fruit jam (Kissan Mango), Tablet (Calcium Sandoz) and Cough syrup (Cheery Cough). 5.0 g of fruit jam/ tablet/ fruit candy or 5.0 ml of fruit syrup / cough syrup was taken and dissolved in Milli Q water and then filtered the mixture and made the volume 10.0 ml. An appropriate aliquot of solution was taken and studied for tartrazine and erythrosine determination by the developed procedure. Amounts of dyes were calculated from the regression equation obtained from calibration curve. The fruit and pharmaceutical samples were spiked with tartrazine and erythrosine. On the basis of calculated value and actual amount of dyes added in fruit samples, recoveries were calculated. Results of analysis are given in (Table III and IV).

Table 1. Amount of synthetic dyes permitted by Ministry of public Health (Thailand), issue 281 (A.D.2547), acceptable daily intake (ADI) values for food safety and countries prohibiting these dyes

Synthetic Dye	Color	Maximum allowance unit (mg/kg of food)	ADI (mg/kg of body weight)	Use prohibited
Tartrazine	yellow	200	0-7.5	Norway, Austria
Erythrosine	red	50	0-4.0	USA, Norway

Table 2.Analysis of calibration curves by HPLC-UV system.

Parameters	Tartrazine	Erythrosine
Regression Equation	0.0129x+0.004	0.0013x+0.002
Correlation Coefficient (r^2)	0.9958	0.9996
RSD (%)	0.004	0.007
LOD (ng/ml)	0.3	0.3
Retention time (min)	1.833	5.050

Table 3. Determination of tartrazine, erythrosine in different spiked food samples by HPLC technique

Sample	Added (ng/ml)		Found (ng/ml)		% Recovery	
	Tartrazine	Erythrosine	Tartrazine	Erythrosine	Tartrazine	Erythrosine
Fruit Syrup	0	0	100	150	-	-
	150	180	251	335	100.6	102.8
	212	245	313.2	400	100.5	102
Fruit Candy	0	0	50	100	-	-
	115	148	160	250	95.7	101.4
	198.5	202.7	249	303	100	100.5
Fruit Jam	0	0	0.90	0.70	-	-
	120	135	121	137	100	100.9
	185	200.7	186.5	201	100.3	99.8

Table 4. Determination of tartrazine, erythrosine in different spiked pharmaceutical samples by HPLC technique

Sample	Added (ng/ml)		Found (ng/ml)		% Recovery	
	Tartrazine	Erythrosine	Tartrazine	Erythrosine	Tartrazine	Erythrosine
Cough syrup	0	0	85.0	52.0	-	-
	103	125	180	179	92.2	101.6
	165	190	251	243	100.6	100.5
	215	245	301	298	100.5	100.4
Tablet	0	0	107	200	-	-
	114	153	225	350	103.5	99.2
	167.5	202	275	401	100.3	99.5
	215	280	321.7	481.3	99.9	100.5

Fig 1. Absoprtion spectra of binary mixture with Cr (III) complexes dissoloved in methanol water at pH 5.5

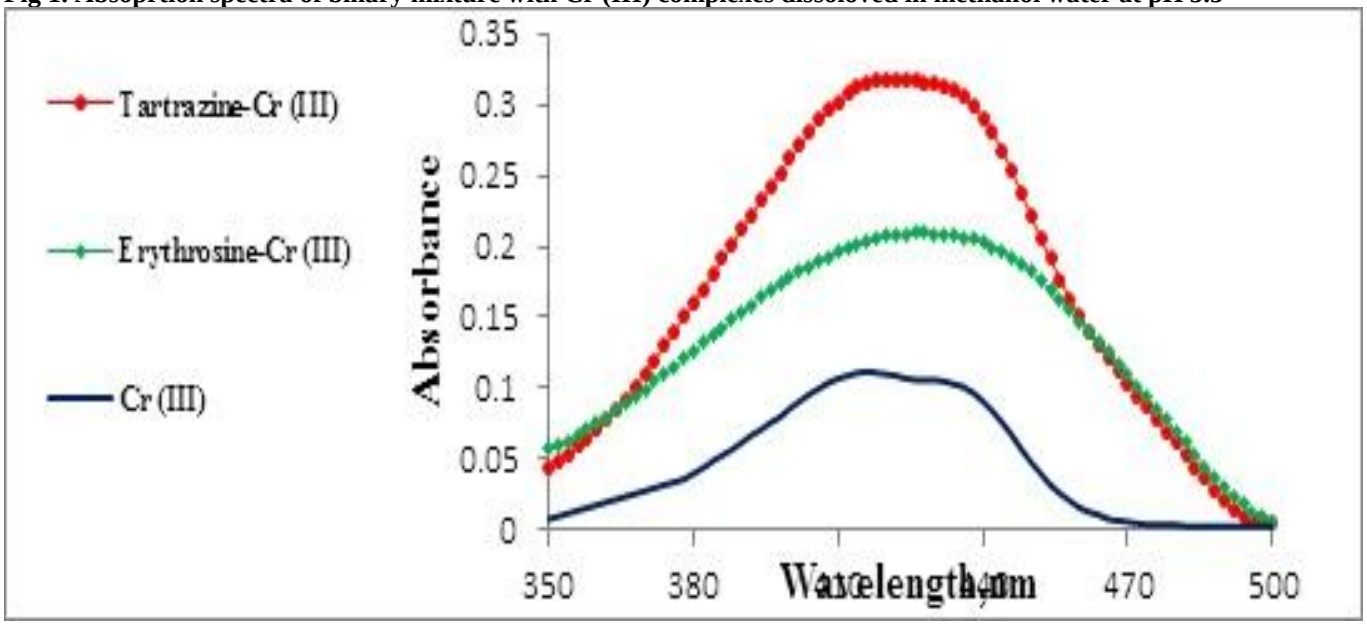
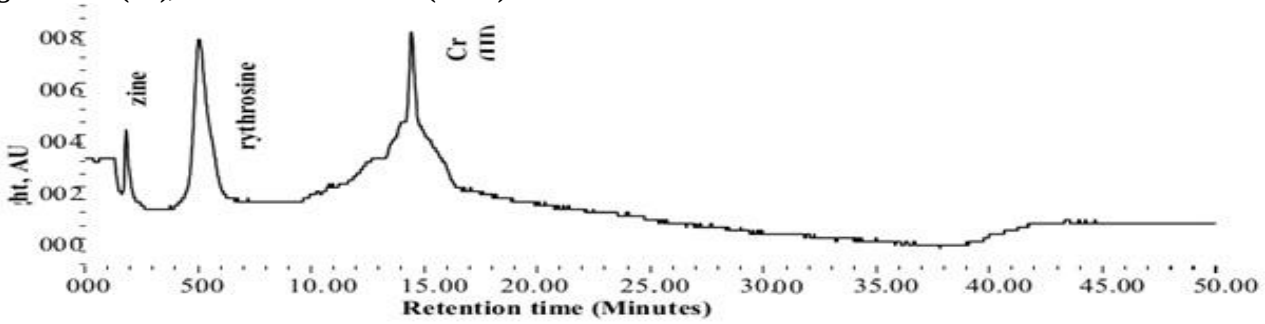


Fig 2. HPLC Chromatogram for mixture of dye complexes containing tartrazine, erythrosine, each at 40.0 ng/ml, 0.5µg/ml of Cr (III), and methanol: water (70:30) at flow rate of 1.0 ml/min and detection at 400.0 nm.



	Name	RT	Area	% Area
1	Peak1	1.833	32419	8.45
2	Peak2	5.050	288258	75.13
3	Peak3	14.438	62984	16.42

CONCLUSION

The separation and determination of binary mixture of synthetic colorants with Cr (III) complexing agent were successful on Intertsil ODS-3V C-18 column by using methanol: water in acetate buffer as a mobile phase. This method is simple and requires minimal cleanup for sample preparation. Detection limits were satisfactory for all real samples. The results showed that this HPLC method was useful for the identification and determination of mixed synthetic dyes in food and pharmaceutical samples.

ACKNOWLEDGMENT

The authors would like to thank the Department of Chemistry, Punjabi University Patiala (PUP) for the financial support.

REFERENCES

1. Altinoz S and Toptan S. Determination of Tartrazine and Ponceau-4R in various food samples by Vierordt's method and ratio spectra first-order derivative UV spectrophotometry. *J.Food Compos. Anal*, 15, 2002, 667-683.
2. Oka H, Ikai Y, Kawamura, N, Yamada M, Inoue H, Ohno T, Inagaki K, Kuno A and Yamamoto N. Simple method for the analysis of food dyes on reversed-phase thin-layer plates. *J. Chromatogr. A*, 411, 1987, 437-444.
3. Gennaro MC, Gioannini E, Angelino S, Aigotti R and Giacosa D. Identification and determination of red dyes in confectionery by ion-interaction high performance liquid chromatography. *J. Chromatogr.A*, 767, 1997, 87-92.
4. Garcia-Falcon MS and Simal-Gandara J. Determination of food dyes in soft drinks containing natural pigments by liquid chromatography with minimal clean-up. *Food Control*, 16, 2005, 293-297
5. Zhang YP, Zhang YJ, Gong WJ, Gopalan AI and Lee KP. Rapid separation of Sudan dyes by reverse-phase high performance liquid chromatography through statistically designed experiments. *J. Chromatogr. A*, 1098, 2005, 183-187.
6. Jaworska M, Szulinska Z, Wilk M and Anuszewska E. Separation of synthetic food colourants in the mixed micellar system: Application to pharmaceutical analysis. *J. Chromatogr. A*, 1081, 2005, 42-47.
7. Perez-Urquiza M and Beltran JL. Determination of dyes in foodstuffs by capillary zone electrophoresis. *J. Chromatogr. A*, 898, 2000, 271-275.
8. Huang HY, Shih YC and Chen YC. Determining eight colorants in milk beverages by capillary electrophoresis. *J. Chromatogr. A*, 959, 2002, 317-325.
9. Giovine LD and Bocca AP. Determination of synthetic dyes in ice-cream by capillary electrophoresis, *Food Control*, 14, 2003, 131-135.
10. Chen QC, Mou SF, Hou XP, Riviello JM and Ni ZM. Determination of eight synthetic food colorants in drinks by high performance ion chromatography. *J. Chromatogr A*, 1998.
11. Michel O, Naeije N, Bracamonte M, Duchateau J, Sergysels R. Decreased sensitivity to tartrazine after aspirin desensitization in an asthmatic patient intolerant to both aspirin and tartrazine. *Annals of Allergy*, 52(5), 1984, 368.
12. Moutinho IL, Bertges LC, Assis RV. Prolonged use of the food dye tartrazine (FD&C yellow no 5) and its effects on the gastric mucosa of Wistar rats. *Brazilian Journal of Biology*, 67(1), 2007, 141.
13. Amin KA, Abdel Hameid H, AbdElsttar AH. Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. *Food ChemToxicol*, 48(10), 2010, 2994.
14. Atayan VZ, SuminaEZ and Shtykov SN. *J.Anal.Chem*, 58, 2003, 7.