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Simultaneous Determination of Colourant Mixtures Used in Pharmaceuticals by Derivative UV-Spectrophotometry

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A derivative spectrophotometric method was developed for quantitative determination of ternary synthetic organic dyes tartrazine (E102;T), indigo carmine (E132, IC) and erythrosine (E127; E), which are under governmental regulations all over the world because of their toxicity and carcinogenity. The main obstacle in the spectrophotometric determination of mixtures of these colourants is the overlapping of their spectra. Since the three colourants studies here present spectral overlapping their simultaneous determination is different when conventional method are used. In this study ternary mixtures of colourants tartrazine, indigo carmine, erythrosine are resolved by using the first and second derivative spectra-zero crossing method without the need for any seperation step. The amplitudes of the first derivative at 453 and 639 nm were used for determination of tartrazine and indigo carmine in the concentration range of 0.5-2.5 and 10.0-80.0 μg/mL, respectively. The amplitude of the second derivative at 561 nm was used for determination of erythrosine in the concentration range 0.2-1.0 μg/mL. This method was successfully applied to commercial pharmaceutical capsule sample containing the three colourants and results were compared with HPLC method.

Key Words: Food colours, Derivative spectrophotometry, Simultaneous determination.

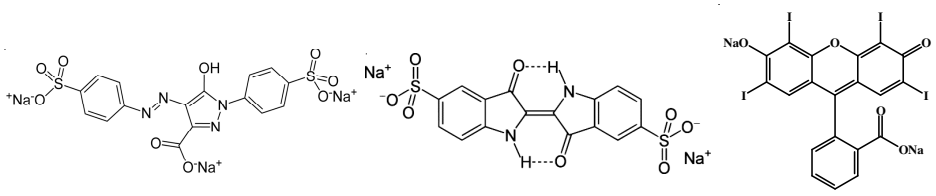
INTRODUCTION

Tartrazine (T), indigo carmine (IC) and erythrosine (E) are synthetic dyes present in pharmaceutial products. The structures of these colourants are shown in Fig. 1.

Over 50 synthetic colourants are used in food, cosmetic and pharmaceutical products all over the world. Synthetic dyes are used under governmental regulations and the kinds and numbers of permitted dyes vary from country to country. Some of these colourants, depending on their long-term and short-term use have potential carcinogenic, toxic and teratogenic effects. A short term effect of food additives and particularly food colours, is hyperactivity in some children, believed to be a form of an allergic response. In the long term some of these foods colours are believed to be carcinogenic.

In Turkey, these colourants have temporary permission of use until studies are completed. The analytical control of these colourants is considerable importance in

food, cosmetic and pharmaceutical industry because of their toxic and carcinogenic potential[1,2]. Tartrazine can induce allergic and asthmatic responses in sensitive people[3]. Toxicological properties of indigo carmine is reported in literature[4]. The colour content of foods, pharmaceuticals and cosmetics must be included in the list ingredients.



Tartrazine Indigo carmine Erythrosine

Fig. 1. Chemical structures of tartrazine, indigo carmine and erythrosine

Many analytical methods have been developed for the codetermination of colourants in mixture. These methods include thin layer chromatography (TLC)[5-7], thin layer chromatography (TLC) with UV/vis spectrophotometry[8], column chromatography[9], high performance liquid chromatography[10-13], spectrophotometry[14-21], capillary electrophoresis[22,24], differential pulse polarography[25], voltammetry[26], mass spectrophotometry[27] and various combinations of these techniques. One of the classical problems of analytical chemistry is the simultaneous determination of two or more compounds in a sample without prior separation of the components.

Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands by using the first or higher derivatives of absorbance with respect to wavelength. This technique offers an alternative approach to the enhancement of sensitivity and specificity in mixture analysis. In consists of calculating and plotting one of the mathematical derivatives of a spectral curve. The derivative transformation does not increase the information content of a spectrum but it permits discrimination against broad band interferences arising from turbidity or non-specific matrix absorption[28].

In recent years derivative spectrophotometry and partial least squares (PLS) and principal companent regression (PCR) multivariate calibration methods have been widely used for the determination of dyes in foods, cosmetics and phamaceutical products and satisfactory results were reported in a review with 450 references[28].

No spectrophotometric method has been published for the simultaneous determination of tartrazine, indigo carmine and erythrosine in the literature. The aim of this work, is to develope simple and accurate spectrophotometric method for the simultaneous determination of tartrazine, indigo carmine and erythrosine in a three-component mixture without the need for prior separation. The developed method was applied to determine the content of Coldeks capsules and synthetic mixtures.

EXPERIMENTAL

Spectrophotometric determinations were made using a Shimadzu UV-2450 PC UV-visible spectrophotometer connected to a baseline computer.

All chemicals and solvents were analytical reagent-grade unless otherwise indicated. Tartrazine (T), indigo carmine (IC), erythrosine (E) were obtained from Hayat Kimya Turkey. Coldeks capsules, containing tartrazine, erythrosine and indigo carmine were procured from Turkey Pharmacy. Standard solution 100 µg/mL of tartrazine , 1000 µg/mL of indigo carmine and 25 µg/mL of erythrosine were prepared from the pure product by dissolving appropriate weights in distilled water and stored in refrigerator at 4 ºC and darkplace. Working solutions in the range 0.2-80.0 µg/mL were prepared freshly every day by an appropriate dissolution of standard solution in distilled water.

Methods: The calibration samples were prepared in 10 mL calibration flasks containing 0.5-2.5 µg/mL of tartrazine, 10.0-80.0 µg /mL of indigo carmine and 0.2-1.0 µg /mL of erythrosine and were diluted to the volume with distilled water. These calibrations were prepared by varying the concentration of the colourant without the presence of the other.

Absorption spectra were recorded between 350-750 nm againts a blank with a scan speed of 500 nm min^{-1} and stored in a disk file. These specta were smoothed and their first and second derivatives were recorded. The tartrazine and the indigo carmine contents were determined from the first derivative spectrum (D_1) by measuring the first derivative signal (peak to zero amplitude) at 453 and 639 nm, respectively and the values were compared with an appropriate calibration graphs. The erythrosine content was determined from the second derivative signals at 561 nm (peak to zero amplitude) and the value compared with an appropriate calibration graphs. A validation set of three colourant were also prepared in the same conditions for calibration solutions.

Preparation of sample solution: Initially, qualitative assays were done for the detection of colourants in capsules by paper chromatography. Water-dissolved samples and standard dyes were applied to chromatographic papers and developed in the mobile phase of *n*-butyl alcohol-ethyl alcohol-water (1:1:1 v/v/v). R_f values were compared and dyes were detected[5,7,29].

For the tartrazine, indigo carmine and erythrosine determination in Coldeks capsules, the content of 20 capsules was emptied out. 1.2825 g empty capsules was accurately weighted and transferred into a 25 mL volumetric flask. 10 mL of distilled water added and the mixture was shaken for 5 min. Next, the volume was made up to the mark with distilled water, mixed thoroughyl and filtered The first 5 mL portion of the filtered was discarded. Such prepared solutions were next treated according to the procedures as given in methods section.

RESULTS AND DISCUSSION

Fig. 2 shows the zero-order absorption spectra of aqueous solutions of tartrazine, indigo carmine, erythrosine. indigo carmine can be approximately determined by

direct absorbance measurement at 500 nm But the others can not be determined accurately because of the overlapped spectra. Therefore, derivative spectra were used for the satisfactory resolution of this problem.

Derivative spectra of synthetic dyes: The first derivative spectra of tartrazine, indigo carmine and erythrosine solutions are shown in Fig. 3. It is clear that indigo carmine can be determined in the presence of erythrosine and tartrazine at 639 nm (working zero-crossing wavelengths of erythrosine and tartrazine) and also tartrazine can be determined in the presence of erythrosine and indigo carmine at 453 nm. The second derivative spectra of tartrazine, indigo carmine and erythrosine solutions are shown in Fig. 4. Erythrosine was determined in the presence of tartrazine and indigo carmine at 561 nm by second derivative method. At these wavelengths absorbance signals of the mixture are consistent with those obtained for each dye separately.

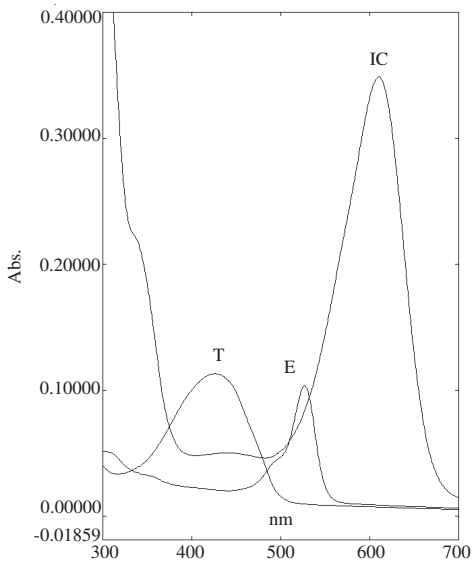


Fig. 2. Absorption spectra of 2.5 µg/mL tartrazine, 10 µg/ mL indigo carmine, 1.0 µg/ mL erythrosine

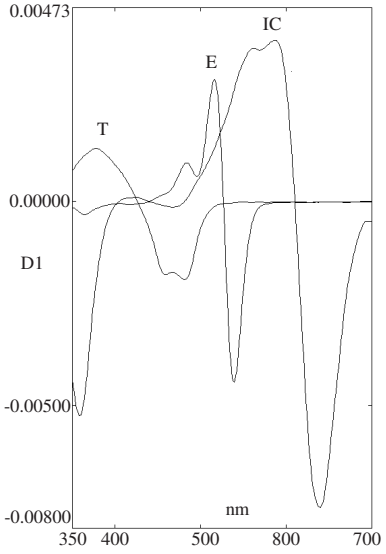


Fig. 3. First derivative spectra 2.5 µg/ mL tartrazine, 10 µg/ mL indigo carmine, 1.0 µg/ mL erythrosine

Analytical performance parameters: The calibration graphs were obtained by the range of concentration of tartrazine, indigo carmine and erythrosine (Figs. 5, 6 and 7). The linearity was evaluated by the least square regression method with triplicate determinations at each concentration level. LOD and LOQ were based on the standard deviation of the responce and the slope of the corresponding curve using the following equations: LOD 3s/m; LOQ 10 s/m where s, the noise estimate is the standard deviation of the peak area of the sample and m is the slope of the related calibration graphs. In Table-1, the statistical parameters calculated from the calibration graphs are summarized.

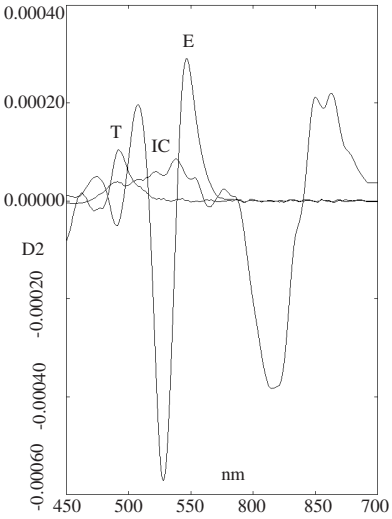


Fig. 4. Second derivative spectra of 2.5 µg/mL tartrazine, 10 µg/mL indigo carmine, 1.0 µg/ mL erythrosine

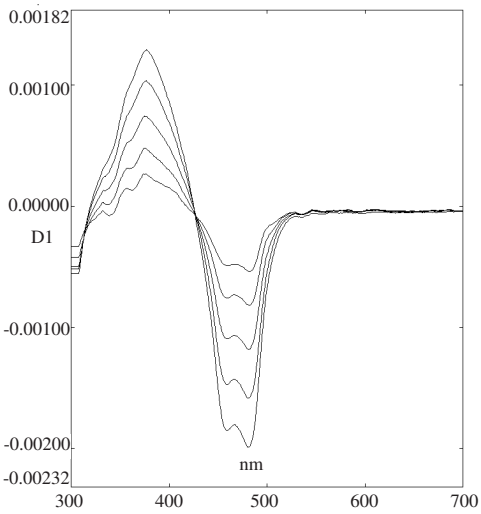


Fig. 5. First derivative spectra of 0.5-2.5 µg/ mL tartrazine

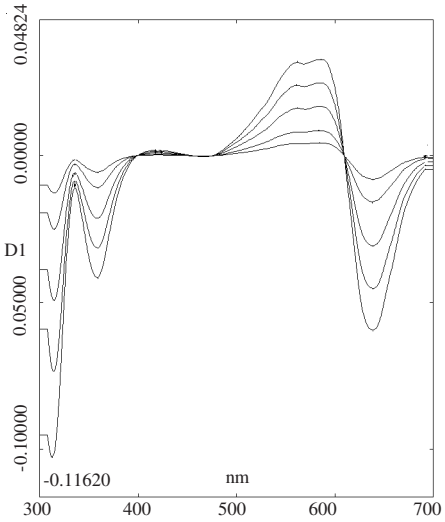


Fig. 6. First derivative spectra of 10.0-80.0 µg/mL indigo carmine

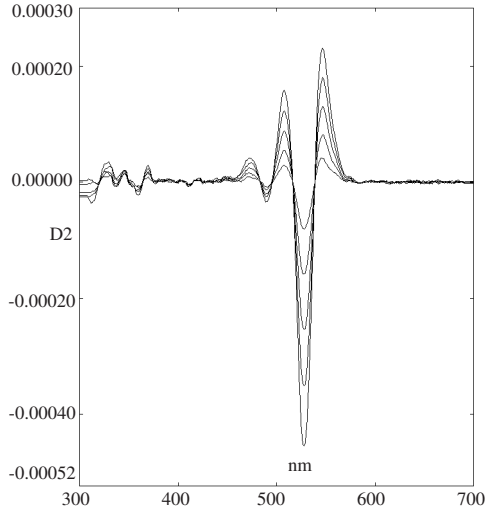


Fig. 7. Second derivative spectra of 0.2-1.0 µg/ mL erythrosine

The validation set consisting of the synthetic ternary mixtures of colourants were performed in 3 times a day and in 4 consecutive days, in order to evaluate intra- and inter-assay accuracy and precision. The values of relative standard deviations and relative errors of tartrazine, indigo carmine and erythrosine are given in Table-2. Satisfactory values were obtained for both the relative standard deviation (RSD) % ranging from 0.41-3.73 and relative error (RE) % ranging from (-0.32)-(4.75).

TABLE-1
STATISTICAL DATA FOR CALIBRATION GRAPHS FOR THE SIMULTANEOUS DETERMINATION OF TARTRAZINE, INDIGO CARMINE AND ERYTHROSINE BY THE DERIVATIVE SPECTROPHOTOMETRIC METHOD

Statistical parameters	Tartrazine	Indigo carmine	Erythrosine
Concentration range (μg/mL)	0.5 - 2.5	10 - 80	0.2 – 1.0
Regression equation	$Y = 1.56 \times 10^{-4} + 4.48 \times 10^{-4}$ C	$Y = 1.16 \times 10^{-3} + 6.81 \times 10^{-4}$ C	$Y = -5 \times 10^{-6} + 6.50 \times 10^{-5}$ C
Correlation coefficient (r)	0.9997	0.9998	0.9912
Limit of detection (μg/mL)	0.01	0.51	0.05
Limit of quantification (μg/mL)	0.37	1.72	0.18

TABLE-2
EVALUATION OF INTRA-DAY AND INTER-DAY ACCURACY AND PRECISION

	Tartrazine (μg/mL)			Indigo carmine (μg/mL)			Erythrosine (μg/mL)		
	1	1.5	2	20	40	60	0.4	0.6	0.8
Intra-assay	0.97	1.45	1.98	19.91	40.13	59.83	0.38	0.58	0.77
RSD (%)	3.17	2.76	1.79	1.25	0.76	0.45	3.23	2.95	2.46
RE (%)	3.00	3.30	1.00	0.45	-0.32	0.28	4.50	4.16	3.70
Inter-assay	1.01	1.46	1.99	19.87	40.14	59.66	0.38	0.57	0.77
RSD (%)	3.73	2.42	2.54	1.12	0.72	0.41	3.01	2.88	2.92
RE (%)	-1.00	2.6	0.50	0.65	-0.35	0.56	4.75	4.16	3.90

RE: Relative error; RSD: Relative standard deviation.

Determination of samples: The proposed method was applied to calculate the contents of the three colourants in pharmaceutical product. In order to test accuracy of the proposed method, tartrazine, erythrosine and indigo carmine contents in pharmaceutical product were determined simultaneously by HPLC. In this method C_{18} column and the mobile phase system consisted of methanol and 0.025 M KH_2PO_4/K_2HPO_4 pH 7 buffers are used. The flow rate was 1.5 μg/mL. The assay results obtained by both methods were statistically compared at the 5 % level. As shown in Table-3 there were no significant differences between the mean values and precisions of the two methods.

TABLE-3
COMPARISON OF RESULTS FOR THE QUANTITATIVE DETERMINATION OF TARTRAZINE, INDIGO CARMINE AND ERYTHROSINE IN "COLDEKS®" CAPSULE SAMPLES BY TWO METHODS

n1 = n2 = 5	Found* (μg/g ± SD)	
	HPLC	Derivative spectrophotometry
Tartrazine	20.70 ± 0.42	21.38 ± 0.75, t = 1.76, F = 3.15
Indigo carmine	901.1 ± 11.4	880.4 ± 18.8, t = 1.33, F = 2.71
Erythrosine	11.16 ± 0.33	10.58 ± 0.60, t = 1.88, F = 3.30

*: Mean value of five determinations. Tabulated t-value at the 95 % confidence level is 2.77. Tabulated F-value at the 95 % confidence level is 6.39.

Conclusion

The derivative spectrophotometric method was developed to assay tartrazine, indigo carmine and erythrosine in pure forms and in pharmaceutical formulations. The proposed method does not involve elaborate sample preparation protocols and interference from excipients. The results obtained have been validated by the HPLC method. The developed method offers the following advantages when compared to current HPLC methods *i.e.*, minimizing cost and time of analysis, no pH- adjustment and inexpensive instrumentation. The proposed method can be used for the routine analysis of food colourant and commercial product containing the tetrazine, indigo caramine and erythrosine colourants.

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