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# Simultaneous Determination of Tartrazine and Sunset Yellow in Cosmetic Products by First-derivative Spectrophotometry

**Luis Fermín Capitán-Vallvey, Natalia Navas Iglesias, Ignacio de Orbe Payá,** and **Ramiro Avidad Castaneda***

Department of Analytical Chemistry, University of Granada, E-18071 Granada, Spain

**Abstract.** A spectrophotometric method for the simultaneous determination of Tartrazine (TT) and Sunset Yellow (SY) in cosmetic products has been developed. An extraction process was carried out using methylene chloride and the colouring matters were measured in the aqueous phase formed, the other components of the sample remaining in the organic phase. The applicable concentration ranges were 0.5–10 μg/ml TT and 0.5–12 μg/ml SY. The detection limits were 26 and 11 ng/ml and the relative standard deviations were 1.0 and 0.9% for TT and SY, respectively. The method was applied to the determination of both compounds in cosmetics.

**Key words:** first-derivative spectrophotometry, cosmetics, Tartrazine–Sunset Yellow mixture determination.

Tartrazine [TT] (C.I. 19140) and Sunset Yellow [SY] (C.I. 15985) are two synthetic colorants widely used as additives in bath gels, colognes and other cosmetic products. Several papers have shown that the contact of these chemicals with the human body can produce asthma, allergies and other discomforts in sensitive people, which is why the amounts of these compounds in cosmetic products must be controlled.

For the analysis of such colorants, conventional and derivative spectrophotometric analytical techniques have been used [1–4]. More recently, derivative and ratio spectra derivative spectrophotometry have been proposed for the determination of binary and ternary mixtures of Tartrazine, Sunset Yellow and Quinoline Yellow in foods [5–8]. These procedures do not require any prior separation step.

Electroanalytical techniques also provide appropriate methods for the determination of different colorants. Differential pulse voltammetry with solid electrodes or a dropping mercury electrode has been proposed [9–11]. Likewise, high performance liquid chromatography (HPLC) has been used for detection and determination of these compounds.

However, in most cosmetics only one, two or three colorants are present and that is why, taking into account rapidity and economy, spectrophotometric methods can be most appropriate for determining colorants in cosmetics. The determination of these compounds in cosmetics presents an additional difficulty. Usually the fragrances, essences and other components presents in colognes, bath gels, after-shave lotions, etc. hinder the direct analysis of these samples by conventional or derivative spectrophotometry.

In this paper we propose a simple, economic and sensitive method for the simultaneous determination of TT and SY, singly or as a mixture, in colognes, bath gels and after-shave lotion by first-derivative spectrophotometry. The absorbance of the colorants is directly measured in the aqueous phase formed after extraction with methylene chloride for separation from the other potentially interfering organic components.

## Experimental

### *Apparatus and Software*

A Perkin-Elmer Lambda-2 spectrophotometer (with 1-cm cell) connected to an IE 486 computer fitted with Perkin-Elmer PECSS software and a Hewlett-Packard Laser III printer were used for all

* To whom correspondence should be addressed

measurements. A Statgraphics software package [12] was used for statistical analysis of the calibration graphs.

*Reagents*

All reagents were of analytical reagent grade unless otherwise stated and reverse-osmosis-quality water was used throughout.

*Stock solution of Tartrazine* (Utter Laboratorios España S.A.) prepared in water at a concentration of 100 μg/ml.

*Stock solution of Sunset Yellow* (Utter Laboratorios España S.A.) prepared in water at a concentration of 100 μg/ml. Both stock solutions were stored in the dark at 4 °C remained stable for at least 1 month.

*Absorbance Measurements*

Absorption spectra were recorded between 400 and 800 nm (at 20.0 ± 0.5 °C) against a blank, with a scan speed of 480 nm/min and stored in a disk file. These spectra were smoothed, with 13 experimental points, and their first derivatives were calculated and recorded using 4-nm intervals. The absorbances were measured in a 1-cm cell at 484.5 nm for TT and at 527 nm for SY against a blank solution, since at these wavelengths the first-derivative absorbances of SY and TT are respectively zero.

*Procedure*

To an appropriate aliquot of sample solution containing between 0.5 and 10.0 μg/ml of TT and between 0.5 and 12 μg/ml of SY in a 50-ml calibrated flask, appropriate volumes of water and ethanol were added to maintain in all cases 70% (v/v) of ethanol. Immediately after, 20 ml of this solution were transferred to a 100-ml separatory funnel and 16 ml of $CH_2Cl_2$ were added. After shaking for 1 min, the hydroalcoholic phase containing the colorants was transferred to a 10-ml calibrated flask and made up to the mark with ethanol. Absorbance measurements were performed against a blank solution of ethanol (96%). Calibration graphs were constructed in the same way using TT and SY solutions of known concentration. Three measurements were carried out for each standard, and the lack-of-fit test applied to prove the linearity of the calibration graphs. For analysis of real samples the standard additions method was used.

*Sample Preparation*

10 g of real sample (bath gel or shampoo), or 2 ml in the case of cologne, were placed in a 25-ml calibrated flask and made up to the mark with water. 6 ml of this solution were mixed with 14 ml of ethanol and then extracted as above before the absorbance was measured.

Prior to the determination of the colorants in commercial cosmetic samples their identification by TLC [13] was carried out.

## Results and Discussion

### *Spectral Characteristics*

The absorption spectrum of TT shows only one absorption band between 400 and 523 nm, with the absorption maximum at 429 nm. The absorption spectrum of SY shows an absorption band starting before 400 nm and concluding at 560 nm, with an absorption maximum at 485 nm. As can be seen in Fig. 1, the absorption spectra of both colorants overlap considerably, hindering the resolution of their mixtures by conventional spectrophotometry. In principle, SY could be determined at 515–520 nm in the presence of TT, but the accuracy is compromised by the sensitivity to wavelength changes; TT could not be determined at the same time.

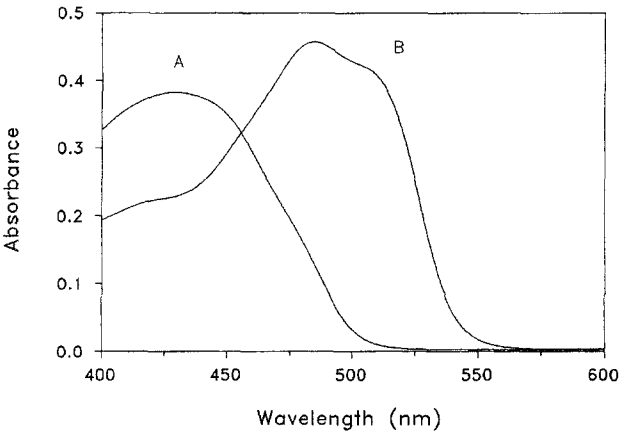


**Fig. 1.** Absorption spectra of (*A*) Tartrazine (5.0 μg/ml) and (*B*) Sunset Yellow (6.0 μg/ml).

This difficulty can be resolved by using the first-derivative spectrum of the mixture of both compounds and applying the "zero-crossing" technique [14, 15]. Hence, the height $h_1(\lambda = 484$ nm) is proportional only to the TT concentration and allows its determination, and the height $h_2(\lambda = 527$ nm) is proportional only to the concentration of SY, allowing its determination (Fig. 2).

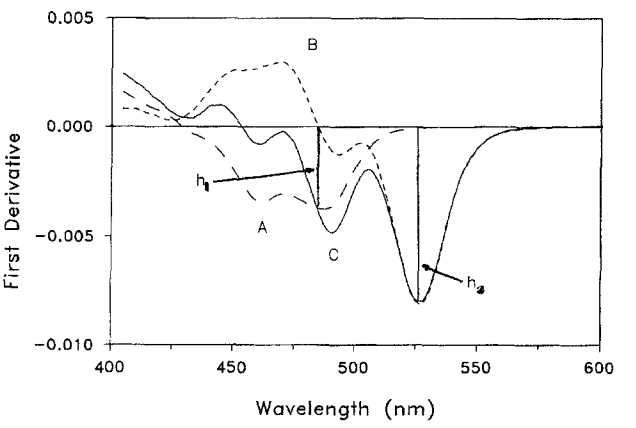


**Fig. 2.** First-derivative absorption spectra of (*A*) Tartrazine (5.0 μg/ml); (*B*) Sunset Yellow (6.0 μg/ml) and (*C*) a mixture of both compounds (5.0 μg/ml for TT and 6.0 μg/ml for SY)

However, the absorption spectra of the essences, fragrances and other additives present in cosmetics products show absorption bands in the same regions as the colorants, hindering the use of conventional and derivative spectrophotometry for their determination. Therefore, we proposed the separation of the interferents by means of extraction with methylene chloride, because the fragrances and essences remain in the organic phase formed when the sample is shaken with $CH_2Cl_2$.

*Other Experimental and Instrumental Parameters*

In order to check the influence of other experimental parameters we have to distinguish between those that affect the extraction process and those affecting the absorbance measurements. Experimental and instrumental parameters such as percentage of ethanol and scan speed do not affect the measurements of absorbance. The extraction step is mainly influenced by the percentage of ethanol and the amount of $CH_2Cl_2$.

To reduce the noise levels on the derivative spectra, a smoothing function based on the Savitzky-Golay [16, 17] method for 13 experimental points and an interval of 4 nm was obtained. A scan speed of 240 nm/min was selected as optimum after verifying that this parameter hardly affected the derivative signal obtained, because the differentiation is obtained not electronically but numerically.

In order to test the influence of other experimental conditions on the extraction step, the shaking time, the amount of $CH_2Cl_2$ and the percentage of ethanol were studied. If the ethanol/water ratio is less than 80:20 (v/v) and the methylene chloride volume $\geqslant 80\%$ of the volume of the ethanol/water mixture, two phases are formed [18]. At the $EtOH/H_2O/CH_2Cl_2$ proportions (v/v) suggested above, the hydroalcoholic phase contains the colorants while the other additives remain in the organic phase (ethanol/methylene chloride). However, the absorbance of the system is influenced by the proportion of ethanol/water used in the extraction step. As Fig. 3 shows, for values between 60:40 and 70:30 ($EtOH/H_2O$ v/v) the absorbance remained constant. Finally, the selected shaking time was 1 min, because after 30 s the process of extraction was not influenced by this parameter.

*Analytical Parameters*

The calibration graphs are linear in the concentration ranges 0.5–10.0 μg/ml for TT and 0.5–12.0 μg/ml for SY. The adjustment of these analytical curves was carried out by linear regression, with the lack-of-fit test applied to probe its linearity (two replicates of each standard), as suggested by the Analytical Methods Committee [19]. Table 1 shows other analytical parameters.

In order to test the mutual independence of the analytical signals for TT and SY, i.e., to show that the signal produced by each colorant is independent of the concentration of the other, four calibration graphs were obtained for standards containing between 0.5 and 12.0 μg/ml of SY in the presence of 0.0, 0.5, 3.0 and 10.0 μg/ml of TT. Following the same procedure four calibration graphs were constructed for standards containing between 0.5 and 10.0 μg/ml of TT in the presence of 0.0, 0.5, 4.0 and 12.0 μg/ml of SY.

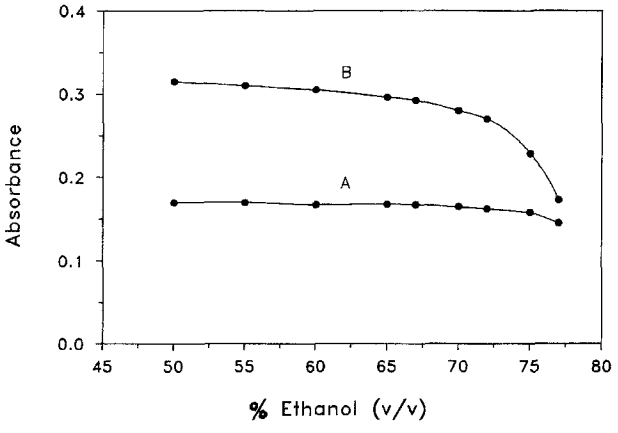


**Fig. 3.** Influence of the $EtOH/H_2O$ (v/v) proportion in the extraction process. (*A*) TT (5.0 μ/ml) and (*B*) SY (6.0 μ/ml)

**Table 1.** Analytical parameters

| Parameters | Tartrazine | Sunset Yellow |
|---|---|---|
| Signal measured | $^1D_{484}$ | $^1D_{527}$ |
| Intercept | $-2.5 \times 10^{-4}$ | $-1.45 \times 10^{-3}$ |
| Slope | 0.0146 | 0.0263 |
| $P_{lof}$[a] (%) | 31.0 | 30.0 |
| LDR[b] (μg/ml) | 0.5–10.0 | 0.5–12.0 |
| Detection limit [21] (ng/ml) | 26 | 11 |
| Quantification limit [22] (ng/ml) | 87 | 36 |
| RSD[c] (%) | 1.0 | 0.9 |

[a] Probability level of the lack-of-fit test.
[b] Linear Dynamic Range.
[c] Relative standard Deviation.

**Table 2.** Calibration graphs of TT (0.5–10.0 μg/ml) and SY (0.5–12.0 μg/ml) in mixtures

| Compound determined | Co-existing compound | μg/ml | Slope | Intercept |
|---|---|---|---|---|
| Tartrazine | Sunset Yellow | 0 | 0.0146 | $-2.50 \times 10^{-4}$ |
| | | 0.5 | 0.0146 | $-2.51 \times 10^{-4}$ |
| | | 3.0 | 0.0146 | $4.79 \times 10^{-4}$ |
| | | 12.0 | 0.0149 | $18.00 \times 10^{-4}$ |
| Sunset Yellow | Tartrazine | 0 | 0.0263 | $-1.45 \times 10^{-3}$ |
| | | 0.5 | 0.0257 | $1.47 \times 10^{-3}$ |
| | | 3.0 | 0.0259 | $1.39 \times 10^{-3}$ |
| | | 10.0 | 0.0255 | $2.61 \times 10^{-3}$ |

**Table 3.** Recovery study of TT and SY mixtures in synthetic mixtures

| Sample | Sunset Yellow | | | Tartrazine | | |
|---|---|---|---|---|---|---|
| | Taken (μg/ml) | Found[a] (μg/ml) | Recovery (%) | Taken (μg/ml) | Found[a] (μg/ml) | Recovery (%) |
| 1 | 1.0 | 0.93 | 93.0 | 1.0 | 0.99 | 99.0 |
| 2 | 1.0 | 0.97 | 97.0 | 3.0 | 2.82 | 94.0 |
| 3 | 1.0 | 0.97 | 97.0 | 6.0 | 5.46 | 91.0 |
| 4 | 3.0 | 2.94 | 98.0 | 1.0 | 1.01 | 101.0 |
| 5 | 3.0 | 2.96 | 98.7 | 3.0 | 3.02 | 100.6 |
| 6 | 3.0 | 2.96 | 98.7 | 6.0 | 5.98 | 99.7 |
| 7 | 5.0 | 5.01 | 100.2 | 1.0 | 1.02 | 102.0 |
| 8 | 5.0 | 5.09 | 101.8 | 3.0 | 3.05 | 101.6 |
| 9 | 5.0 | 5.01 | 100.2 | 6.0 | 6.08 | 101.4 |

[a] Average of three determinations.

Table 2 summarizes the results, from which can be deduced the independence of the analytical signals produced by each colorant, because the slopes of the corresponding calibration graphs remained constant in all instances.

### *Statistical Calculations*

The IUPAC detection limits [20, 21] and quantification limits [22] were also calculated (Table 1). The repeatability of the method, given as relative standard deviations (RSDs), was determined for three series of ten samples, containing respectively 3.0 μg/ml TT, 6.0 μg/ml SY and both 3.0 μg/ml TT and 6.0 μg/ml SY. The RSDs were 1.0% for TT, 0.9% for SY and 1.0% for TT and SY mixed.

### *Applications of the Method*

In order of check the accuracy of the method, a recovery study was carried out on synthetic samples containing different amounts of TT and SY. Table 3 shows the results obtained, proving that the accuracy of the method was acceptable in all instances. The standard additions method to obtain the calibration graphs was used in all cases.

The proposed method was also applied for the determination of TT and SY in different commercial cosmetic products. One bath gel, one cologne and two shampoos were selected as appropriate samples. The bath gel contained Sunset Yellow only, whereas the other products contained both TT and SY. In all instances, prior to their determination, the colorants were identified by thin layer chromatography.

The slopes of the standard additions curves matched those of the calibration graph, *i.e.*, no matrix effect was discerned in any instance. If the colorant concentrations in the diluted real sample were below the lower limits of the linear dynamic ranges of the

**Table 4.** Determination of TT and SY in commercial cosmetics

| Cosmetics | TT (μg/ml)[a] | SY (μg/ml)[a] |
|---|---|---|
| Cologne | 8.8 | 7.5 |
| Bath gel | np[b] | 9.4 |
| Shampoo 1 | 10.3 | 4.1 |
| Shampoo 2 | 6.4 | 1.2 |

[a] Average of three determinations.
[b] Not present.

calibration graphs, good results were obtained by adding constant amounts of both colorants to the sample for analysis. This was usually the case for shampoo and bath gel, because these samples were aqueous and must be diluted to give the 70% (v/v) ethanol/water solution to carry out the extraction process. The results are summarized in Table 4.

## Conclusions

This paper provides a practical and simple application of first-derivative spectrophotometry for simultaneous determination of Tartrazine and Sunset Yellow in colognes, bath gel and shampoo without previous separation and using conventional instrumentation.

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