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# A rapid HPLC method for the analysis of water soluble synthetic pigments in soft drinks

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**Abstract** The goal of the present contribution was the development of a liquid chromatographic method for the determination of water soluble synthetic pigments in soft drinks. The method consists in the direct injection of the sample (after filtration) in a HPLC system with diode array detection on a reverse phase column. The HPLC method was validated by specific performance criteria, such as the linearity of the calibration curve, recovery percentage and estimation of the measurement uncertainty. The results obtained for the determination of two of the most used synthetic colorants, FD&C Yellow 5 and Orange Yellow S on real soft drink samples demonstrated that the described HPLC method is a useful technique for analyzing synthetic dyes, rapid and simple, with an appropriate uncertainty of the measurements.

*Keywords:* synthetic pigments, FD&C Yellow 5, Orange Yellow S, HPLC, soft drinks analysis.

## 1. Introduction

Food pigments have been used for centuries to keep or to improve the perceived flavour and color of the food, drinks and cosmetics. Two categories of pigments are used in the food industry: synthetic pigments and natural ones. Synthetic dyes are more stable than the natural ones during the manufacturing and storage food processes, they being not so sensitive to the degradation factors. On the negative side, synthetic pigments can cause adverse toxicological effects, being considered as unhealthily substances for humans [1]. For this reason, the international regulations require the presence of synthetic colorants, such as FD&C Yellow 5 (E 102) and Orange Yellow S (E 110) (commercial named Tartrazine and Sunset Yellow) to be declared in food, drug and cosmetic products. The Council Directive 94/34 [2] has also stipulated a reduced number of permitted synthetic colorants for use in foodstuffs, under certain conditions and limits in use.

FD&C Yellow 5 (YELL5), Color Index 19140, and Orange Yellow S (OYS), Color Index 15985, are two synthetic azo water soluble dyes, very wide used as yellow soft drinks pigments. Their molecular formula are $C_{16}H_9N_4Na_3O_9S_2$, trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)-H-pyrazole-3-carboxylate for YELL5 and $C_{16}H_{10}Na_2O_7S_2N_2$, disodium 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalene-sulfonate for OYS, with the chemical structures presented above (Fig. 1).

Both YELL5 and OYS are synthetic permitted colorants in food processing by legislations, the maximum accepted level by Romanian law [3] being harmonised with those provided by the Council Directive 94/34 [2]. The limit value in food of YELL5 is 100 ppm and of OYS 50 ppm, respectively, when individually and no more than 100 ppm where they are used in binary mixtures.

An impressive number of determination methods have been developed in order to analyze food synthetic dyes, including YELL5 and OYS, either separately or in combination with other colorants. The majority of the analytical methods for the determination of synthetic colorants are in the range of spectrophotometric methods and chromatographic ones [4-7]. When only the identification of food colorants is intended, the qualitative methods are mostly based on paper chromatography and thin–layer chromatography [8-9]. Therefore, the development of modern and powerful analytical techniques able to separate, to identify and to

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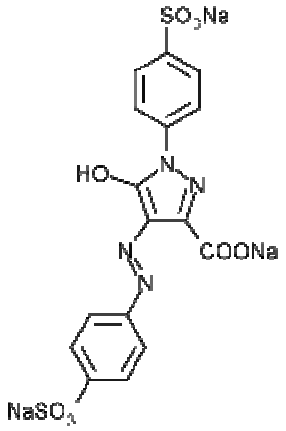
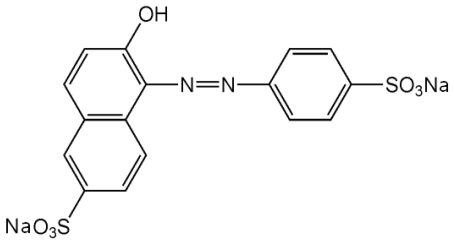
quantify different synthetic food pigments it became very important.




FD&C YELLOW 5




ORANGE YELLOW S

**Fig. 1** The chemical structures of YELL5 and OYS

In this study, YELL5 and OYS were identified and quantitatively determined in soft drinks, using a high performance liquid chromatograph system Agilent Series 1100 with diode array UV detector (G1315B DAD DE 43626997), equipped with autosampler (G1313A DE43631050), degasser (G1379A JP 54426011), column compartment (G1316A DE 43649671) and a quaternary pump (G1311A DE 43634416)

This HPLC method was in house validated and the specific performance criteria have been determined: linearity of calibration curve, recovery and the measurement uncertainty, following the methods described in the references [10-12].

## 2. Experimental

*Reagents*

All reagents used were of chromatographic purity grade and all solutions were prepared using bidistilled water. FD&C Yellow 5 and Orange Yellow S were purchased from SIGMA-Aldrich (Germany, purity 90%), methanol and acetonitrile were from Merck (Germany), Chromasolv purity. Monopotassium phosphate and disodium phosphate were from Scharlow (France).

*Mixed stock standard solution (1000 mg/L) preparation:*

Weigh 0.1000 g ± 0.0005 g of each colour, dissolve in bidistilled water and dilute up to 100 mL with the same solvent. Label and refrigerate the solution at 2 – 8 ºC temperature.

*Mixed calibration colour standard preparation:*

After equilibrating to room temperature, dilute 0.05, 0.1, 0.2, 0.5 and 1.0 mL of stock solution to 10 mL with blank sample (lemon juice) in order to obtain the mixed calibration standards of 5, 10, 20, 50 and 100 mg/L (prepare on day of use).

Injection volume was 10 µL.

The separations of the two colorants were performed on reverse column Hypersil 5 µm MOS $C_8$ (phenomenex/ 00G-0151-E0/413245-1) of 250 mm in length and 4.60 mm in diameter. The column temperature was set at 30ºC, using two mobile phases in a gradient elution system.

*Mobile phase:*

In the present method the mobile phase consists from two components, denoted with I and II:

Component I – 0.01 M sodium phosphate and 0.88 mM potasium phosphate, pH 8.0 ± 0.2, prepared by dissolution of 1.4196 ± 0.0010 g sodium phosphate and 0.1200 ± 0.0010 g potassium phosphate to 1 L; discard after one week;

Component II – Acetonitrile: Methanol 1:4, discard after six months.

The mobile phases gradient programme includes three steps: isocratic elution at 5%A:95%B (5 min), linear gradient from 5%A:95%B to 45%A:55%B (15 min), linear gradient to recover initial conditions of 5%A:95%B.

The flow-rate of the eluent was established at 1.0 mL/min.

*Washing mobile phase:*

In order to keep the column at optimal parameters a washing solution of 70% water: 30% methanol is used at the end of the experiments.

*Sample preparation*:

The soft drink samples are degassed using an ultrasonic bath for 10 minutes and the pH is adjust to 6.50 (± 0.10) by drop wise addition of a NaOH solution (10 %). After that, the liquid sample of soft drink is vacuum filtered on typical micro filtration membrane with the pore size of 0.45 µm.

## 3. Results and Discussions

In order to chose the most appropriate detection wavelengths, the UV-Vis absorption spectra have been obtained for YELL5 and OYS, using 10 mg/L standard solutions of both colorants, separately and in mixture (5 mg/L YELL5 + 5 mg/L OYS). The absorption spectra for 350-600 nm range are represented in Fig. 2, where the spectral region between 200-350 nm was not represented because this region is strongly affected by a high background. As it can be seen from the registered spectra, the maximum absorption wavelengths are situated in visible range, at 454 nm for YELL5 and 482 nm for OYS, respectively. Therefore, for the absorbance measurements the spectral region between 430-490 nm is appropriate, because that contains the main spectral information for the two analytes. The optimal detection wavelength for chromatographic analysis in further experiments was set at 470 nm ± 2 nm, having a reference wavelength of 520 nm ± 2 nm.

The identification of the colour peaks on chromatograms were realised by comparison of the retention times with those of the colour standards.

For a precise identification of the peaks, the retention times should not differ by more than ±5% of the working standards, and this condition was very well preserved on further experiments.

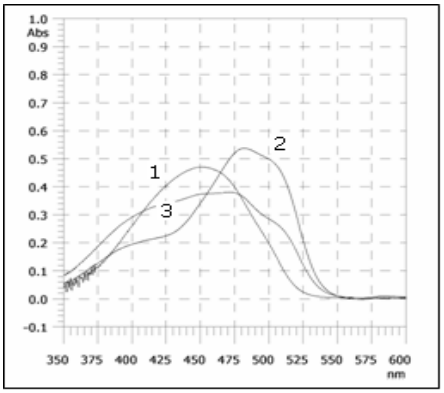
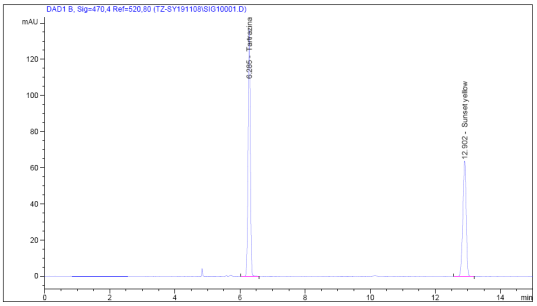



**Fig.2.** UV-Vis spectra of standard solutions of FD&C Yellow 5, 10mg/L (1), Orange Yellow 10mg/L (2) and mixture of FD&C Yellow and Orange Yellow S, (3).

The calibration curves for YELL5 and OYS were obtained using various concentrations of mixed YELL5 and OYS standards six fold. The linearity of the curve with the formula y=mx was 5-150 mg/L for both colorants and the slope of the curve was 12.54988. The value of the correlation coefficient is 0.9999, confirming the high degree of linearity in the mentioned concentration range.

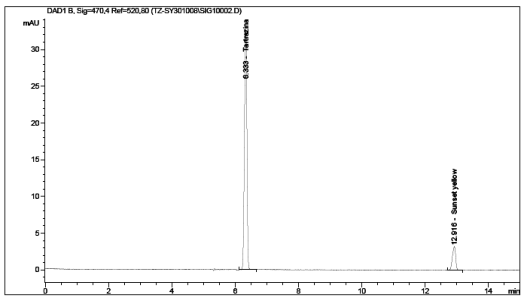
In Fig.3 is represented the chromatogram for mixed standard solution containing 50 mg/L of YELL5 and 50 mg/L of OYS.




| RetTime [min] | Type | Area [mAU*s] | Amt/Area | Amount [mg/L] | Grp | Name |
|---|---|---|---|---|---|---|
| 6.198 | VV | 658.35150 | 7.58240e-2 | 49.91883 | | Tartrazina |
| 12.638 | VV | 464.73419 | 1.07265e-1 | 49.84967 | | Sunset yellow |
| Totals : | | | | 99.76849 | | |

**Fig.3.** The chromatogram of mixed standard solution containing 50 mg/L of YELL5 and 50 mg/L of OYS.

A positive gaseous lemon soft drink sample in YELL5 and OYS is exemplified in Fig.4.




| RetTime [min] | Type | Area [mAU*s] | Amt/Area | Amount [mg/L] | Grp | Name |
|---|---|---|---|---|---|---|
| 6.333 | BB | 179.66972 | 7.58240e-2 | 13.62327 | | Tartrazina |
| 12.916 | BB | 22.70354 | 1.07265e-1 | 2.43529 | | Sunset yellow |
| Totals : | | | | 16.05857 | | |

**Fig 4.** Chromatogram of gaseous lemon soft drink sample in YELL5 and OYS.

No interferences in the determination of YELL5 and OYS from sweeteners, flavors or preservatives (citrate, sodium benzoate, saccharin, aspartame and sorbic acid) possible presents in the matrix soft drink samples have been detected under the experimental conditions of the present HPLC method.

The recovery percentage for the HPLC method under study was determined as one of the most important analytical parameter and in order to establish it one soft drink sample was fortified with both colorants. Good values of recovery has been obtained, ranging for YELL5 determination between 92.50 -101.67% and for OYS determination between 92.14-104.24%.

The effect of individual uncertainty components (linearity of the calibration curve, detection limit, quantification limit, recovery, repeatability, reproducibility, ruggedness, stability) on the expanded uncertainty measurement was also investigated. The calculated value of the extended combined uncertainty was 1.24 mg/L, which represents an appropriate value for the HPLC method.

Details about the validation parameters of this method will be the subject of a further paper.

## 4. Conclusions

A liquid chromatographic method with diode array detection was developed and optimized for the identification and determination of two permitted synthetic pigments in soft drinks in Romanian market, FD&C Yellow 5 and Orange Yellow S. The amount of the sample required for the analysis is very small, with a limited sample pre-treatment. The method was validated in house and overall, the critical analytical parameters revealing the appropriateness of the described method for the purpose of food authenticity.

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