

# Determination of food colorants in food with HPLC-UV

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## Abstract

This application note presents a method using high performance liquid chromatography for the determination of food colorants in sweets. Using stationary phases with 5 µm particle size make the method suitable for simple HPLC systems.

## Introduction

Foods nowadays must have a pleasing appearance. Therefore food dyes are increasingly used in food production. The use of food dyes is regulated in the EU Regulation Nr. 1333/200 [1]. The labeling as well as the approval of food dyes for certain food groups must be observed. The use in food production and the labeling of food colorants are controlled by the official food control.

The determination of food colorants in food with HPLC-UV can be used instead of thin layer chromatography and offers a simple and safe analysis of these analyte group.

## Compounds of interest

E number	Analyte
E102	Tartrazine
E104	Quinoline Yellow WS
E110	Sunset Yellow FCF
E122	Azorubine
E123	Amaranth
E124	Ponceau 4R
E127	Erythrosine
E129	Allura Red AC
E131	Patent Blue V
E132	Indigo carmine
E133	Brilliant Blue FCF

Table 1: Overview of the analytes.



## Subsequent analysis: HPLC-UV

### Chromatographic conditions

#### Column:

EC 150/2 NUCLEODUR® π<sup>2</sup>, 5 µm, (REF 760624.20)

#### Eluent A:

H<sub>2</sub>O (ultrapure) + 20 mM ammonium acetate

#### Eluent B:

Methanol

#### Gradient:

Hold 10 % B for 0.5 min, in 6.0 min to 60 % B, in 4.0 min to 80 % B, hold 80 % B for 2 min, in 1 min to 10 % B, hold 10 % B for 10 min

#### Flow rate:

0.3 mL/min

#### Temperature:

30 °C

#### Injection volume:

10 µL

#### Detection:

UV: 430 nm, 520 nm, 590 nm

## Detection wavelengths and concentration of analyte mixture:

Analyte	Detection wavelengths [nm]	Concentration in water (β) [µg/mL]
Tartrazine	430	1.9
Quinoline Yellow WS	430	2.1
Sunset Yellow FCF	430	1.7
Azorubine	520	1.7
Amaranth	520	2.8
Ponceau 4R	520	1.9
Erythrosine	520	1.7
Allura Red AC	520	1.5
Patent Blue V	590	1.9
Indigo carmine	520	1.9
Brilliant Blue FCF	590	1.8

Table 2: Detection wavelengths and concentrations of analyte mixtures for the analysis of food colorants.

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## Chromatogram of a mixture of 11 food colorants

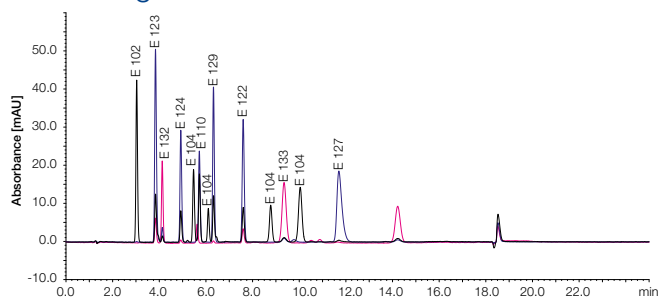


Figure 1: Chromatogram of HPLC-UV analysis of a mixture of 11 food colorants, detection wavelength: black 430 nm, pink 520 nm, blue 590 nm.

Analyte	Retention time [min]
E 102	3.04
E 123	3.85
E 132	4.12
E 124	4.96
E 104	5.46
E 110	5.72
E 104	6.12
E 129	6.33
E 122	7.63
E 104	8.81
E 133	9.39
E 104	10.09
E 127	11.97

Table 3: Retention times of HPLC analysis.

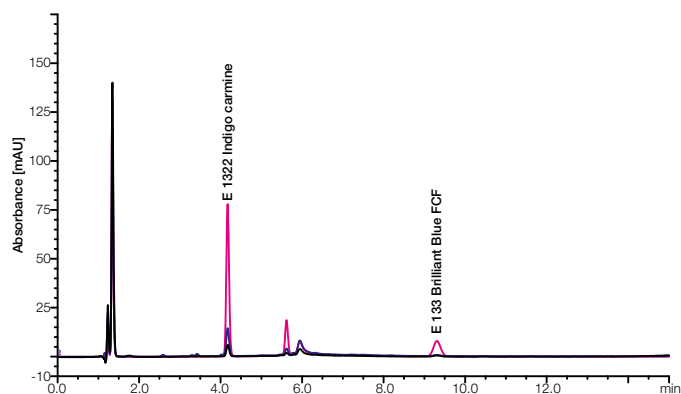


Figure 2: Chromatogram of HPLC-UV analysis of colored chewy (Skittles), detection wavelength: black 430 nm, pink 520 nm, blue 590 nm, identified colorants E 132 and E 133.

### Sample preparation (Chewy "Skittles")

- Weigh out 5 g of homogenized sample
- Dissolve sample in 5 mL water
- Stir mixture for 1 hour
- Centrifuge at 14000 rpm for 5 min at room temperature
- Sample extract ready for injection

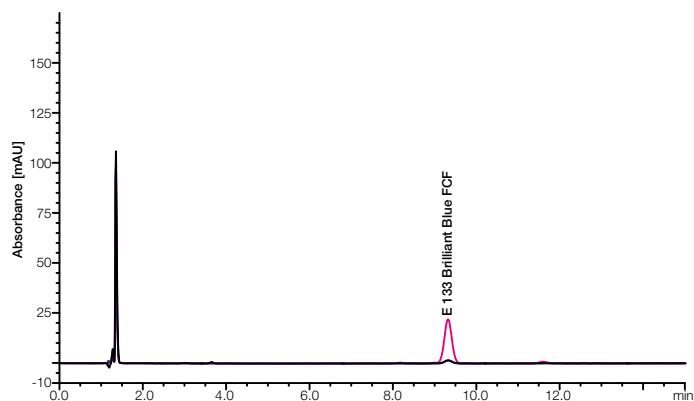


Figure 3: Chromatogram of HPLC-UV analysis of colored decor butterflies, detection wavelength: black 430 nm, pink 520 nm, blue 590 nm, identified colorant E 133.

### Sample preparation (Decor butterflies)

- Weigh out 1 g of homogenized sample
- Dissolve sample in 5 mL water
- Stir mixture for 1 hour
- Centrifuge at 14000 rpm for 5 min at room temperature
- Sample extract ready for injection

### Results and discussion:

The results show that the proposed method for the determination of food colorants in food is a suitable alternative to thin layer methods. Sample preparation presented in that application note is fast and simple. HPLC-UV allows identification and quantification with specific wavelengths of the food colorants in one chromatographic run.

### References

1. A. Domke, R. Großklaus, B. Niemann, H. Przyrembel, K. Richter, E. Schmidt, A. Weißenborn, B. Wörner, R. Ziegenhagen, Use of vitamins in foods -Toxicological and nutritional physiological aspects, BfR 2004.
2. M. Glanzig, ERNÄHRUNG/NUTRITION, 2006, 30/9, 363-368.
3. The European Union's Food Supplements Directive of 2002.

### Additional Information

The following applications regarding „Determination of food colorants in food with HPLC-UV“ and further applications can be found on our online application database at [www.mn-net.com/apps](http://www.mn-net.com/apps)  
HPLC: MN Appl. No. 128210

### Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 760624.20, EC 150/2 NUCLEODUR® π<sup>2</sup>, 5 µm  
REF 702293, Screw neck vials N 9, 1.5 mL  
REF 702107, N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red

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