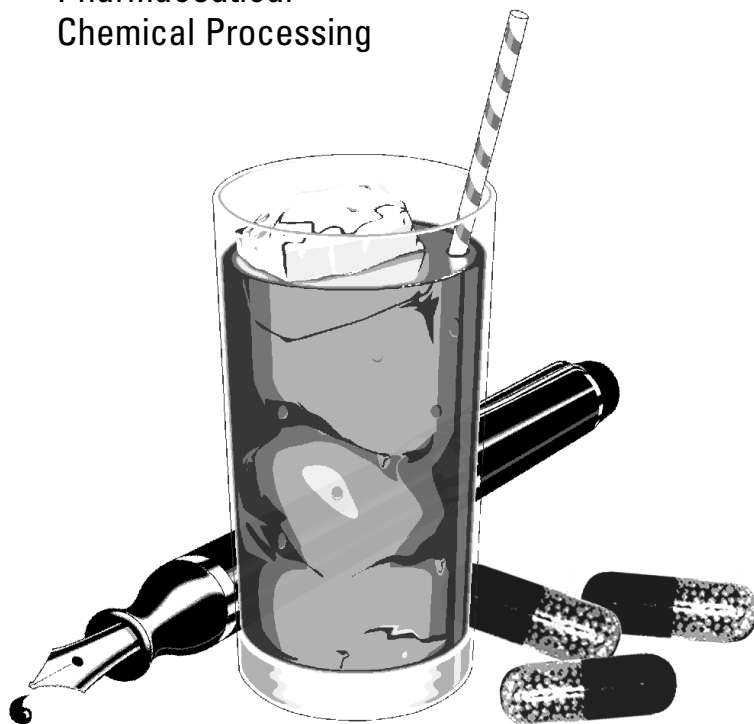


Sensitive Analysis of Synthetic Colors using HPLC and Diode- Array Detection at 190–950 nm

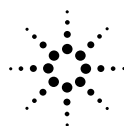
Application Note

Food and Flavors
Pharmaceutical
Chemical Processing

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In this study synthetic dyes were analyzed using ion-pair, reversed phase chromatography on a special base-deactivated HPLC column. This separation mechanism was chosen to reduce tailing effects of highly polar colors. Detection was performed using a new design of diode-array detector based on two lamps — a deuterium lamp and a tungsten lamp. This ensured highest light output at 190 – 950 nm, which resulted in lowest detection limits over the entire wavelength range. It was possible to analyze blue, black or green colors in the low ng range at their absorption maxima of 600 – 700 nm. Complete spectra, feasible from 190 – 950 nm, facilitated identification with an automated library search.



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Introduction

Synthetic colors are widely used in the food, pharmaceutical and chemical industries.

The regulation of colors and the need for quality control relating to traces of starting products and by-products have forced the development of analytical methods.

Nowadays various HPLC methods are in use, based either in ion-pair reversed phase chromatography or ion-exchange chromatography. UV absorption is the detection method of choice. The UV absorption maxima of colors are very characteristic, starting with maxima at about 400 nm for yellow colors, 500 nm for red colors and at 600 – 700 nm for green, blue and black colors. To analyze all different colors at maximum sensitivity and selectivity, the light output from the detector lamp should be high across the complete wavelength range. For black, green and blue colors, which show absorption maxima at or above 600 nm, this is not possible with conventional UV-Visible detectors based on a one-lamp design. For example, deuterium lamps have their maximum output in the UV range, whereas the visible range shows low light output.

A further analytical problem is the tailing-free separation of dyes. Figure 1 shows that some colors are of more polar nature, which sometimes causes problems even on reversed phases depending on the strength of polarity tailing. This results in worse detection limits and integration problems.

In the following study we evaluated the influence of:

- special deactivated columns on the separation of colors using different mobile phases,
- a newly designed diode-array detection system on limit of detection for colors which absorb at above 500 nm, and
- low noise and complete spectra on identification of sample compounds using automated library search in the low mAU range.

Experimental

For the experiments the Agilent 1100 Series HPLC system was used. The system comprised a low-pressure quaternary gradient pump with vacuum degasser, autosampler, Peltier-regulated column compartment, and diode-array detector with a wavelength range of 190 – 950nm. System control and data evaluation was done through an Agilent ChemStation for HPLC.

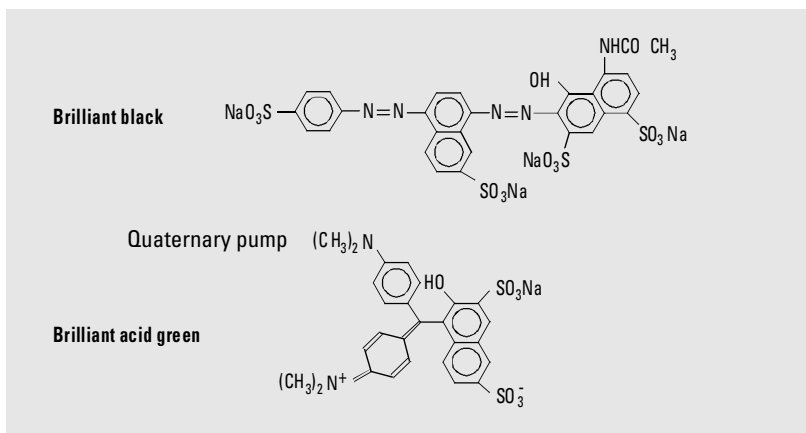


Figure 1
Chemical structure of brilliant black and brilliant green BS

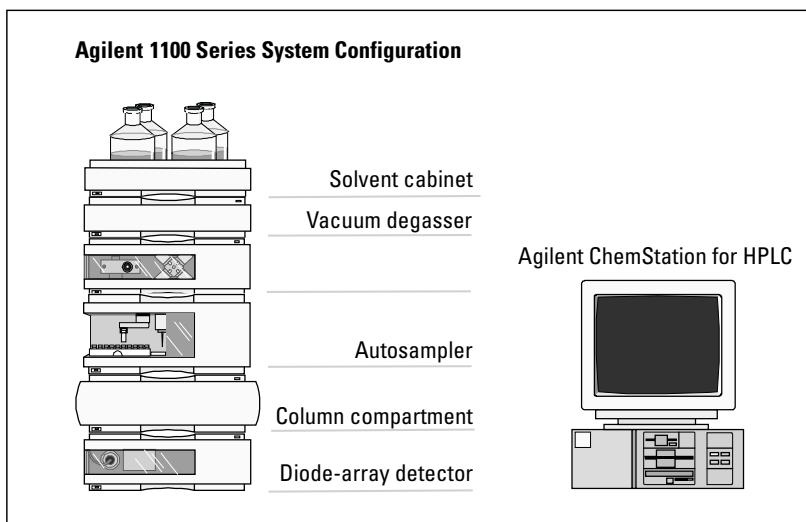


Figure 2
HPLC system used

Results and discussion

Separation of synthetic colors

During method development a special base-deactivated column and four different mobile phases were evaluated. Two mobile phases were simple buffer systems and the other two phases contained ion-pairing reagents (see figure 3). A 125 x 3 mm Hypersil BDS, 3- μ m column (Agilent part number 79926BD-363) was used. The use of 3-mm id. and 3- μ m material allowed optimum flow

rates below 1 ml/min. This saved purchasing disposal costs of solvents. Mobile phases C and D showed tailing for compounds with sulfonic groups. Using ion-pairing chromatography (see figure 3, examples A and B), the separation of colors with different functional groups and different chemical structure was achieved with minimum or no tailing for all different color classes. As a conclusion, we recommend using mobile phases from A.

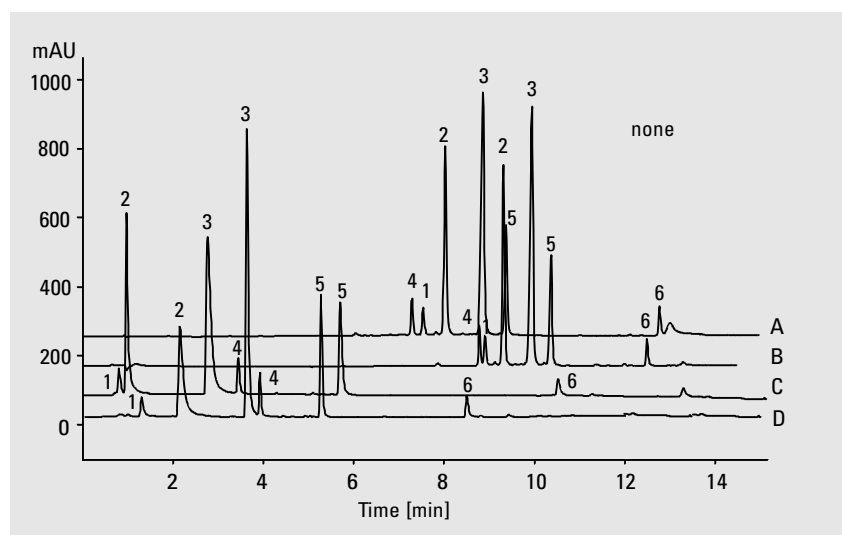


Figure 3
Separation of synthetic colors on BDS column using different mobile phases

Peak	Compound name	Number of sulfonic group
1	E102 Tartrazine	2
2	E123 Amaranth	3
3	E124 Ponceau 4R	3
4	E110 Sunset yellow	2
5	E125 Scarlet red	none
6	E127 Erythrosine	

Table 1
Content of sulfonic groups for colors shown in figure 3

Chromatographic conditions

For the application examples that follow we used mobile phases from A.

Column : 125 x 3 mm Hypersil BDS, 3 μ m (HP part number 79926BD-363)

Mobile phase A: A = 0.01 M NaH_2PO_4 + 0.001 M Tetrabutylammonium-dihydrogenphosphate pH 4.2
B = acetonitrile (ACN)

Gradient for A
start with 15 %, in 10 min to 40 %, in 14 min to 90 %, until 19 min at 90 %, in 20 min to 15 % ACN

Mobile phase B
A = 0.01 M NaH_2PO_4 + 0.001 M Tetrabutyl ammoniumhydrogen sulfate pH 4.8
B = acetonitrile (ACN)

Gradient for B
start with 15 %, in 10 min to 40 %, in 14 min to 90 %, until 19 min at 90 %, in 20 min to 15 % ACN

Mobile phase C
A = 0.01 M ammoniumacetate pH = 4.9, B = ACN

Gradient for C:
start with 7 %, in 10 min to 40 %, in 14 min to 90 %, until 19 min at 90 %, in 20 min to 7 % ACN

Mobile phase D
A = 0.01 M NaH_2PO_4 , pH = 4.3, B = acetonitrile (ACN)

Gradient for D
start with 5 %, in 10 min to 60 %, in 14 min to 90 %, until 19 min at 90 %, in 20 min to 5 % ACN

Stop time 20 min

Post time 4 min

Flow rate 0.8 ml/min

Col. temp. 40 °C

Inject. vol. 1 μ l

Detector
signal A: 254 nm/50 nm (for optimization of separation)
B: 350 nm/20nm,
C: 465 nm/30 nm,
D: 600 nm/40 nm
E: 750/40

Detection of synthetic colors using diode-array UV-visible absorption detector

The diode-array detector used here was equipped with two lamps, a deuterium lamp and a tungsten lamp. This ensured highest light output from 190 to 950 nm and therefore lowest detection limits over the entire wavelength range. The use of 1024 diodes and a programmable slit ensured highest spectral resolution. This gave the following advantages for the analysis of colors:

- acquisition of five signals simultaneously,
- highest sensitivity and selectivity even for blue, green and black colors with absorption maxima above 500 nm,
- complete spectral data up to 950 nm, and
- optimization of signal to noise ratio using different slit width without the need to exchange optical slits mechanically.

Figure 4 shows the complete spectra of a yellow, red and two blue colors.

For each of the analyzed colors characteristic absorption maxima were obtained. The yellow color tartrazine had its maxima at around 400 nm, the red color amaranth absorbed best at about 500 nm, the blue color patent blue had its maxima around 600 nm whereas the darker blue color brilliant blue showed its maxima at 740 nm. This clearly demonstrated that several signals had to be acquired for optimum sensitivity and selectivity for all colors.

The spectra of pure compounds can be stored in spectral libraries and used for later identification of colors in food, paints or pharmaceutical preparations.

That sensitivity is of utmost importance is shown in figure 8. The quality of inks is often determined by the content of traces of other colors, which may be unwanted by-products from production process or which are added to influence the nuance of a color. Therefore the quantitation and identification of trace compounds is as important as the determination of main compounds.

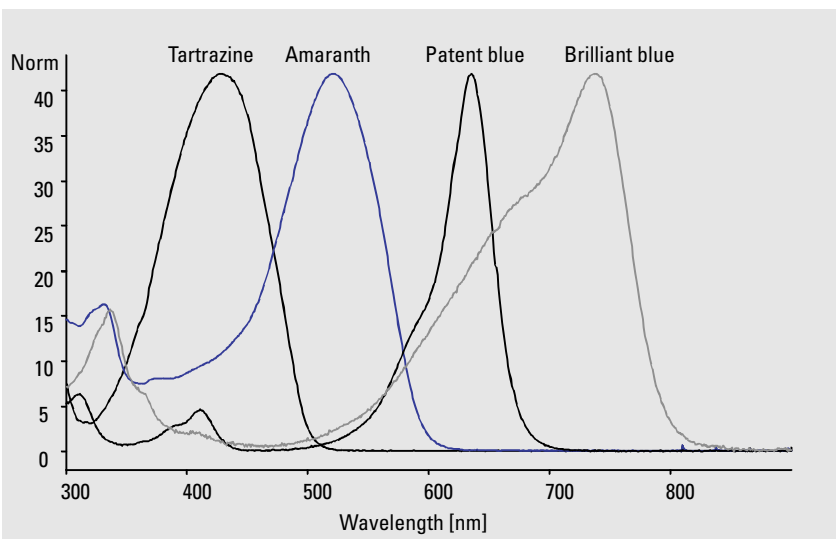


Figure 4
Spectra of different colors

As already demonstrated colors have very characteristic spectra which can be used to identify peaks not only by retention times but also by spectral data. The detector used here allowed identification using spectral data, see figure 5, even in the low mAU absorption range.

The *detection limit* for the evaluated colors, measured at their specific absorption maximum, was in the low ng range.

The *repeatability* of the HPLC method used here was measured using the standard mixture of figure 3. The relative standard deviation for retention times measured over 10 runs was below 0.2 %. For the areas the relative standard deviation was below 1 %.

The *linearity* was evaluated using blue ink color measured at 600 nm. Linearity was given from the low ng up to the low µg range.

Application Examples

Food colors

Colors are vital constituents of foods and probably the first characteristic perceived by the human senses. Today synthetic dyes have widely replaced natural colors. Table 2 lists some of the most frequently used food colors.

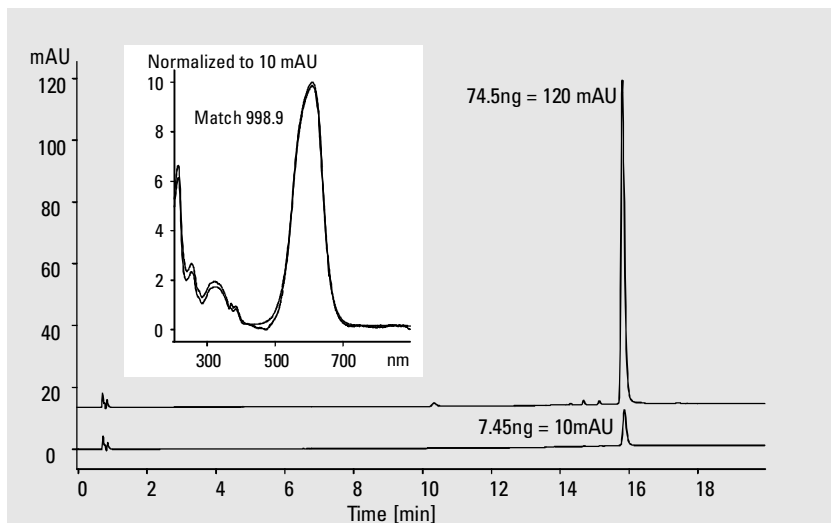


Figure 5
Identification of spectra in the low mAU absorption range for a blue ink color through overlay of trace level sample spectrum with library spectrum

EC	Name	Food, Drug & Cosmetics	CI
E102	Tartrazine	FD&C Yellow No. 5	19140
E103	Chrysoine		14270
E104	Quinoline yellow	FD&C Yellow No. 10	47005
E105	Yellow		13015
E110	Sunset yellow	FCF FD&C Yellow No. 6	15985
E111	Sunset yellow	FD&C Orange No. 2	15980
E122	Azorubine	Carmoisine	14720
E123	Amaranth FD&C	Red No. 2	16185
E124	Ponceau 4R	Ponceau 4R	16255
E125	Scarlet red	Scarlet red	14815
E126	Ponceau 6R		16290
E127	Erythrosine	FD&C Red No. 3	45430
E131	Patent blue V	FD&C Violet No. 1	42051
E132	Indigo carmine	FD&C Blue No. 2	73015
E142	Acid brilliant green	FD&C Green	No. 3
E151	Black PN		28440
	Ponceau SX	FD&C Red No. 4	

Table 2
List of commonly-used colorings in EC and US classifications with color index numbers (CI)

These dyes are used to supplement and enhance the natural colors destroyed during processing or storage, and substantially increase the appeal and acceptability of foodstuffs where no natural colors exist, for example, soft drinks or ice cream.

The usage of synthetic colors is well regulated worldwide, but the regulations differ from one country to the next. To ensure compliance with regulatory requirements, the used colors have to be identified and qualified according to national directives.

As an example of the determination of colors in foodstuffs we analyzed the synthetic colors used for a green carbonated drink — a *woodruff-ade*. The sample was injected directly and the compounds were identified using a library search (figure 6).

The green color was produced by a mixture of quinoline yellow and patent blue. The yellow color quinoline yellow split into four peaks showing an absorption maximum at 410 nm. The blue color patent blue had its maxima at 600 nm.

Colors for pharmaceutical preparations

The pharmaceutical industry uses colors, for example, for tablets, capsules and syrups. Here the intention is not only to improve the optical appearance but also to give more safety to the consumer. For example, different colors may help to avoid errors for a patient who has to take several medicaments.

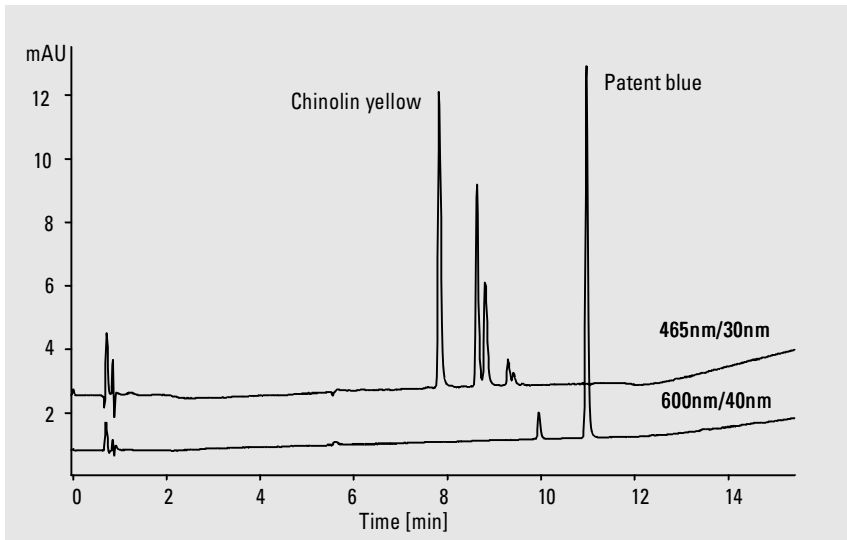


Figure 6
Chromatogram from the analysis of woodruff lemondade

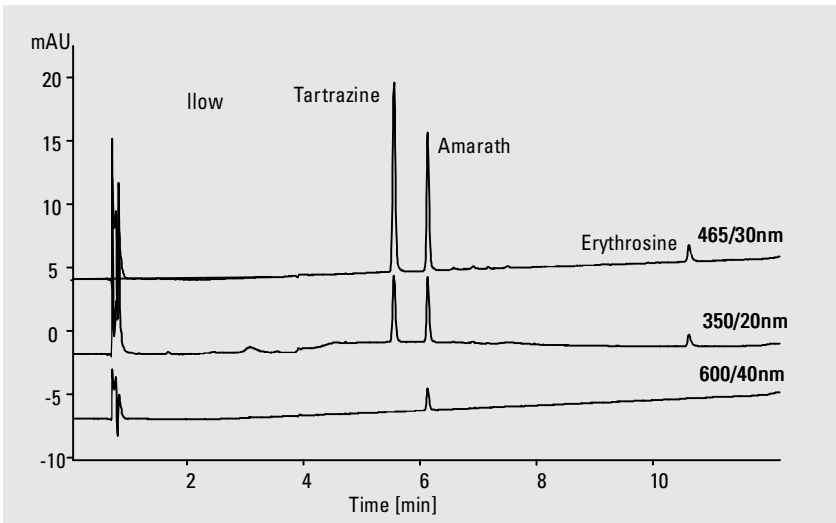


Figure 7
Chromatogram from the analysis of a red capsule

Figure 7 shows the analysis of tablet capsules which were dissolved in water, filtered and injected directly.

The red coloring comprised two red colors erythrosine and amarant and a yellow color tartrazine.

Ink colors

The quality of ink colors relating to color brightness, stability against light and reproducibility of the same colors nuance for years, is determined by the accurate composition of different dyes in different concentrations. Here the right concentration of color traces is as important as the concentration of the main color compounds.

In figure 8 the chromatograms of a blue and a black ink color are overlaid showing that both inks do not only differ in one main compound but also in some trace compounds.

The *blue* color compound in the black ink is producing the main difference between black and blue.

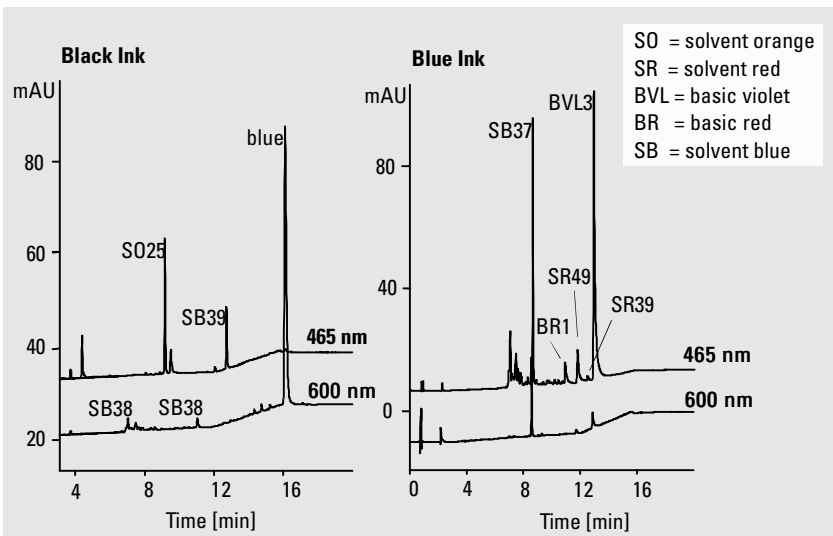


Figure 8
Comparison between a black and a blue ink

Conclusion

In this study we have demonstrated that the separation and detection of synthetic colors can be improved.

Ion-pairing reversed phase chromatography on a special deactivated column allowed the separation of dyes of different polarity with no or only slight tailing.

The UV-visible detection, especially from 400 to 950 nm, gained sensitivity by increasing the light output with a tungsten lamp in addition to a deuterium lamp.

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