Application Note

Food Testing and Agriculture

Analysis of Artificial Colorants in Haw Jelly Using SampliQ WAX Coupled with High Performance Liquid Chromatography (HPLC)

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Abstract

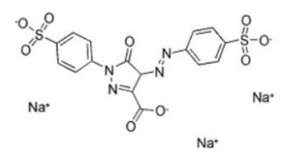
This study developed and validated a method for the quantitative analysis of six artificial colorants in haw jelly candies using the Agilent SampliQ WAX coupled with HPLC analysis. The method delivered a reliable solution with excellent recoveries and reproducibility that can be extended to colorant analysis in other fruit jelly candies.

Experimental

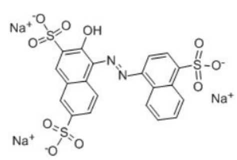
Target analytes

The six target analytes in this application note include Citrine,Amaranth, Carmine, Sunset Yellow, Allura Red, and Brilliant Blue arificial colorants. Figure 1 shows the chemical structures.

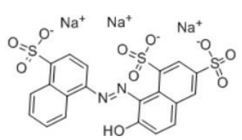
Citrine



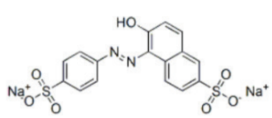
Amaranth



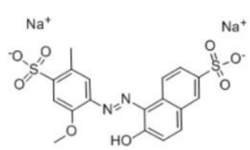
Carmine



Sunset yellow



Allura red



Brilliant blue

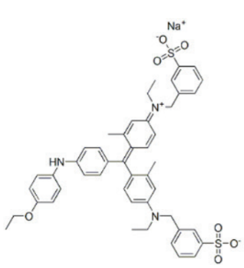


Figure 1. Chemical structures of target analytes.

HPLC conditions

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 100 × 4.6 mm, 2.7 µm (p/n 695975-902)
Flow Rate	1 mL/min
Column Temperature	35 ºC
Injection Volume	10 µL
Detection Wavelength	425 nm (citrine), 500 nm (amaranth, carmine, sunset yellow, allura red), 630 nm (brilliant blue)
Mobile Phase	A) 20 mM ammonium acetate pH = 5 B) ACN
Gradient	Time (min) %A %B 0 100 2 6.0 100 30 11.0 88 90 11.5 88 100 12.0 2 2
Post Time	3 minutes

Instrument method

The samples were run on an Agilent 1260 Infinity II LC system with a diode array detector (DAD). Agilent ChemStation software was used for data acquisition and processing.

Sample extraction

Weigh 2 g of haw jelly piece, then add 20 mg of pectinase and 5 mL water followed by 15 minutes of sonication mixing for enzyme digestion. Add 10 mL of extraction solution (ammonia:water:acetonitrile = 2:48:50), vortex for one minute, then centrifuge for 10 minutes to get the supernatant. Compensate the supernatant to 30 mL with water. Adjust the pH of the sample solution to pH = 6 with formic acid; the sample is now ready for SPE cleanup, following the procedure shown in Figure 2.

Condition a SampliQ WAX (150 mg, 6 mL, p/n 5982-3667) cartridge with 6 mL methanol and 6 mL water.

↓

Load 15 mL of sample (<1 mL/min).

↓

Wash with 6 mL of water and 6 mL of methanol, then dry the cartridge for one minute under vacuum.

↓

Elute 6 mL of methanol with 20% ammonia, then dry the eluent until only water is left at 40 °C under N_2.

↓

Adjust the sample to 1 mL with water, then filter it with a hydrophilic PTFE syringe filter.

↓

The sample is now ready for analysis.

Figure 2. Sample preparation workflow chart.

Results and discussion

Table 1. Method recovery (REC) and RSD.

Analytes	0.5 µg/g Spiking		1 µg/g Spiking		5 µg/g Spiking	
	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
Citrine	99.4	7.9	96.6	2.5	92.2	2.1
Amaranth	95.5	7.4	109.1	3.4	90.7	2.6
Carmine	111.3	6.3	89.3	2.8	88.9	2.0
Sunset Yellow	88.0	8.5	94.0	3.4	90.5	2.1
Allura Red	102.1	7.9	107.3	4.5	90.8	4.4
Brilliant Blue	98.9	8.9	79.7	4.0	88.3	3.3

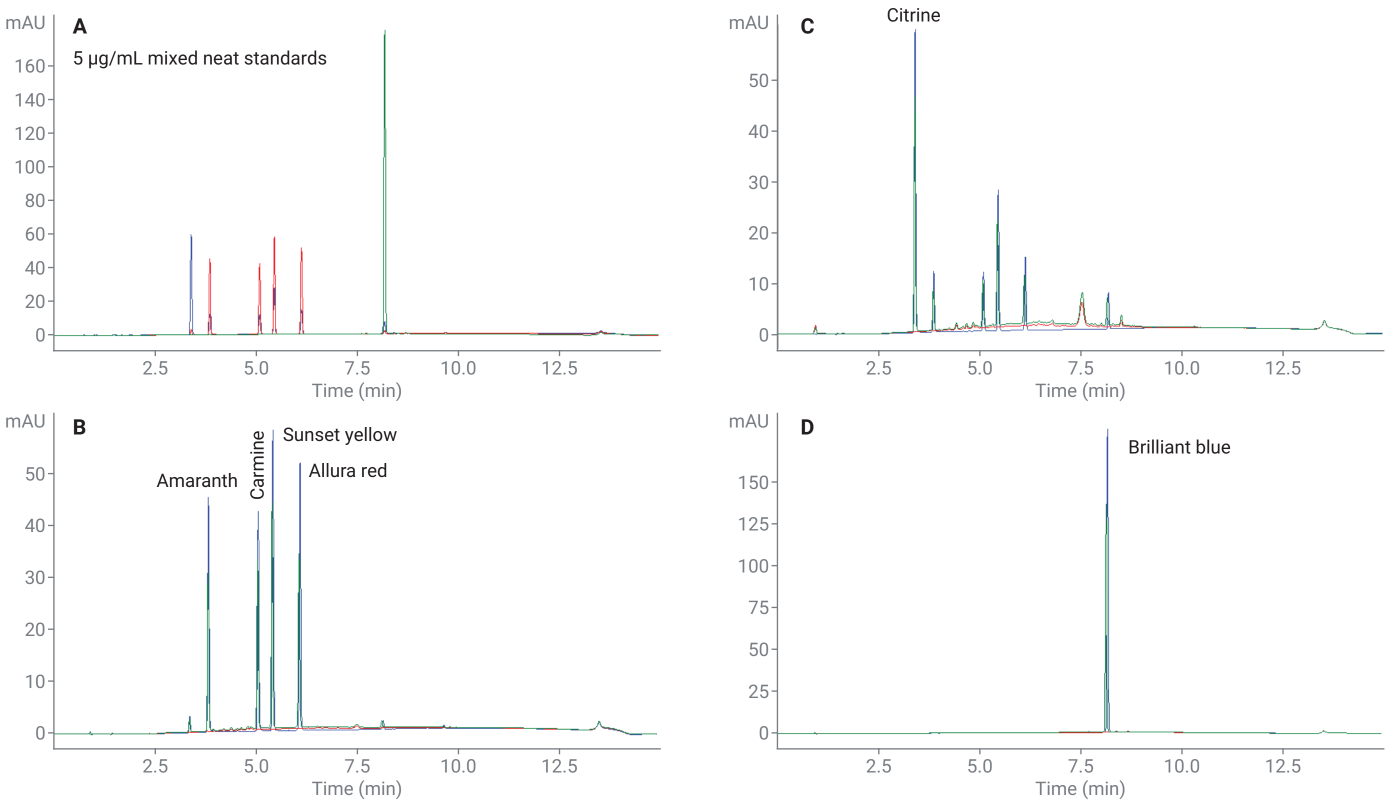


Figure 3. Chromatograms of six artificial pigments in neat standards solution (A) and haw jelly sample (other three figures: blue—neat standard; red—matrix blank; green—pre-spiked sample with 5 µg/g).

Conclusion

This method using Agilent SampliQ WAX SPE cartridges coupled with HPLC for analysis of six artificial colorants in haw jelly candies provided excellent recoveries of approximately 79% to 112%. This method can be a good reference to detect these six artificial colorants in other fruit jelly candies.

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