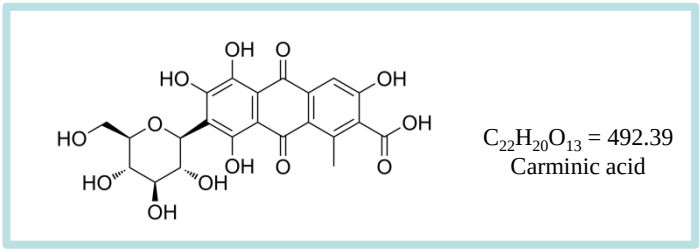
■Analysis Example of Cochineal Extract (Carminic Acid) in Food

AS/LC-019

Cochineal extract is a red dye containing carminic acid, a quinone pigment, as the main component and it is obtained from the cochineal insect (native to Central and South America). Cochineal extract is also found in the List of Existing Food Additives regulated by the Food Sanitation Law (*1) and is widely used in food, drugs, quasi-drugs, and cosmetics.
In the Standard Methods of Analysis for Hygienic Chemistry, TLC is specified as the test method for cochineal extract. However, the analysis method by HPLC is also introduced as a reference method (*2).
Presented here is the analysis of carminic acid in food using HPLC-DAD under the specified analytical conditions. For the qualitative analysis of carminic acid, the DAD absorption spectrum, in addition to the peak retention time, is used for identity confirmation.

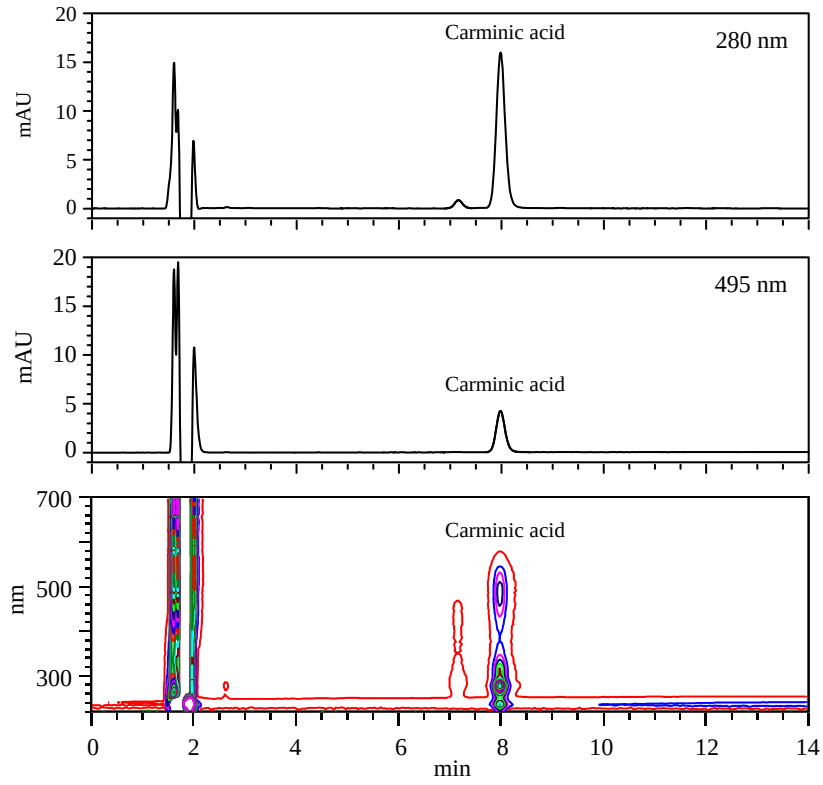
(*1) The List of Existing Food Additives, Notice No. 56 of Food Chemical Division, the Environmental Health Bureau, MHLW (May 23, 1996)
(*2) Standard Methods of Analysis for Hygienic Chemists -with Commentary (2010, The Pharmaceutical Society of Japan)



$C_{22}H_{20}O_{13}$ = 492.39
Carminic acid

[Structural Formula of Carminic Acid]

■ Analysis Example of Carminic Acid Standard Sample

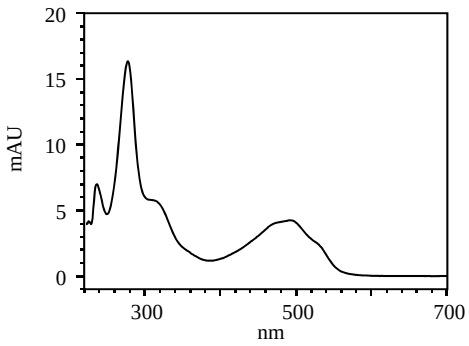


[Analysis Example of Standard Sample (10 mg/L)]

<Analytical Conditions>

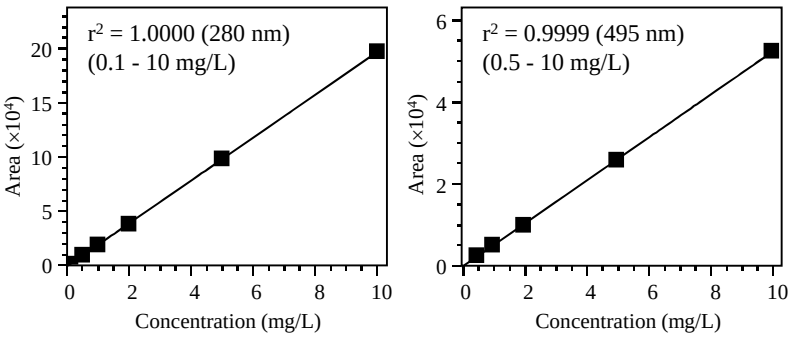
Column	: HITACHI LaChrom C18 (5 μm) 4.6 mm I.D. × 150 mm
Eluent	: 0.1 mol/L citric acid buffer (pH 3.6) / methanol = 75 / 25
Flow rate	: 1.0 mL/min
Column temperature	: 40℃
Detection wavelength	: DAD 220 - 700 nm (280 nm, 495 nm)(*)
Injection vol.	: 20 μL

(*) Standard Methods of Analysis for Hygienic Chemistry describes the analysis example at 495 nm.



[Spectra of Carminic Acid Peak]

■ Linearity



■ Reproducibility (10 mg /L, n = 6)

	Retention time (% RSD)	Area value (% RSD)
280 nm	0.05	0.19
495 nm		0.13

Results indicating good linearity and repeatability were obtained for the carminic acid standard sample.

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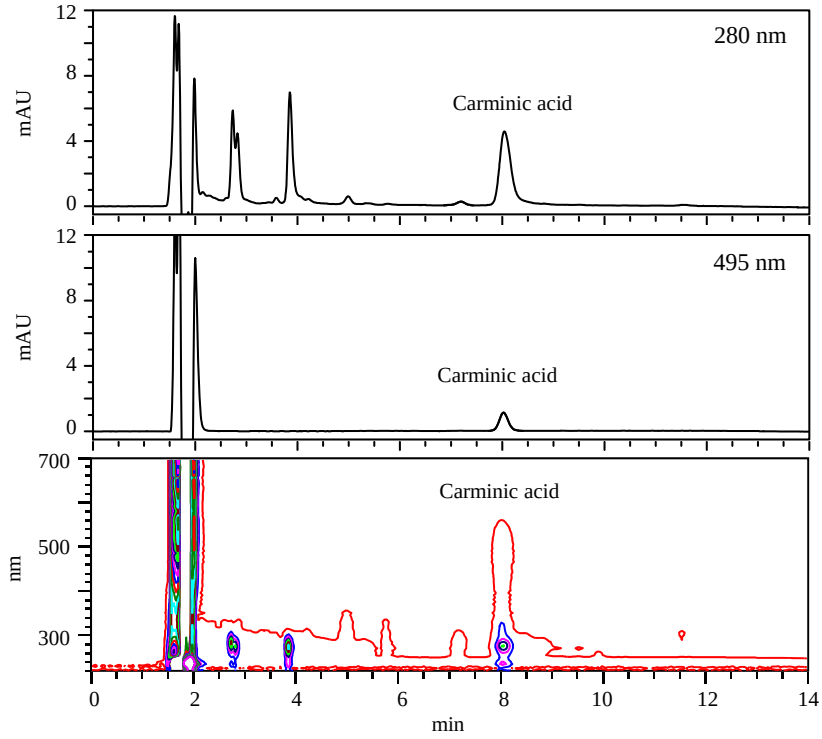
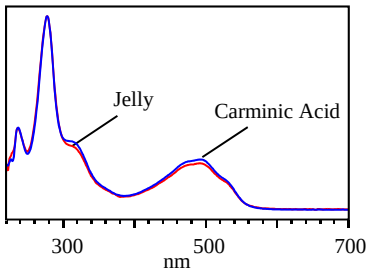


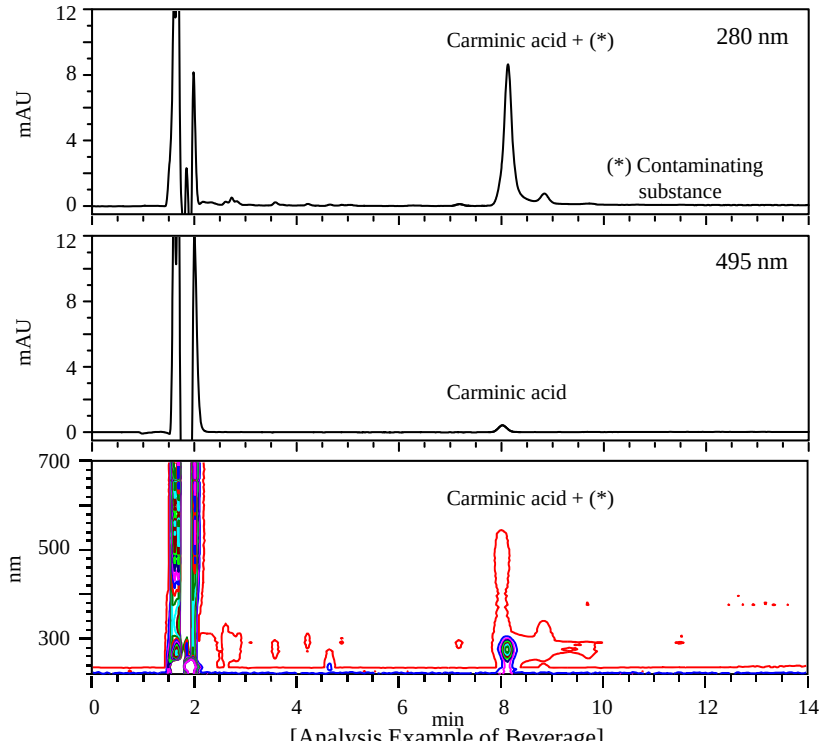
[Analysis Example of Jelly]

<Preparation Method for Jelly>

Jelly Approx. 5 g
| ← 0.1 mol/L HCl,10 mL
Extraction 30 min (Ultrasonic bath)
|
Centrifuge 3000 rpm, 10 min
|
Supernatant
|
10 times dilution (0.1 mol/L HCl)
|
Filtration (0.45 μm filter)
|
Analysis sample (20 μL)



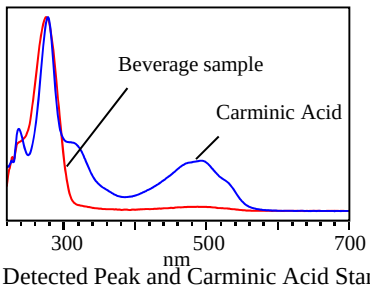
[Spectra of Detected Peak and Carminic Acid Standard Sample]



[Analysis Example of Beverage]

<Preparation Method for Beverage>

Beverage (containing fat) 1 mL
| ← 1 mL of 5 % perchloric acid
Centrifuge 10000 rpm, 10 min
|
Supernatant 1 mL
| ← 1 mL of hexane
Shake 1 min
|
Centrifuge 10000 rpm, 10 min
|
Dilute the bottom layer (aqueous layer) to 10 times with 0.1 mol/L HCl
|
Filtration (0.45 μm filter)
|
Analysis sample (20 μL)



[Spectra of Detected Peak and Carminic Acid Standard Sample]

By using DAD, the absorption spectra of the peaks detected in the standard sample and food sample can be compared. It was found that the spectral shape obtained from the beverage is different from the standard spectral shape while the spectrum of the jelly sample corresponded with that of the standard. Thus, the peak detected in the beverage is considered to be the result of the overlap of carminic acid and other components. In addition, when the purity of this peak was determined by using the purity check function, the purity was confirmed to be low. As described above, the qualitative analysis capability can be strengthened by using DAD.

Main system configuration: Chromaster 5110 Pump, 5210 Autosampler, 5310 Column Oven, 5430 DAD

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.
The system is for research for use only, and is not intended for any animal or human therapeutic or diagnostic use.