



Analysis of Antioxidant Ethoxyquin in Spices

Ethoxyquin (EQ) is used as an antioxidant for the purpose of preserving the colors of spices, such as paprika and chili powder, in the USA and Canada, but its use as a food additive is prohibited in Japan.

EQ can be analyzed by a UV detector (254 nm). However, by using a fluorescence detector, EQ can be analyzed more selectively and with high sensitivity, eliminating the interference of other substances in a sample.

In this study, the Chromaster HPLC system (with FL detector), spices were analyzed for EQ in accordance with the Methods of Analysis in Health Science with Commentary 2010, Methods for Food Additives (The Pharmaceutical Society of Japan).



High Performance Liquid Chromatograph Chromaster®

Analysis of EQ Standard Solution

- ✓ Standard solution: 10 mg of EQ was weighed and dissolved in acetonitrile to make a volume of 100 mL (100 µg/mL).
- ✓ Standard solutions for calibration curve: Measure 0, 2, 4, 6, 8, and 10 mL of the standard solution and added mobile phase to make a volume of 10 mL (0, 20, 40, 60, 80, 100 µg/mL)

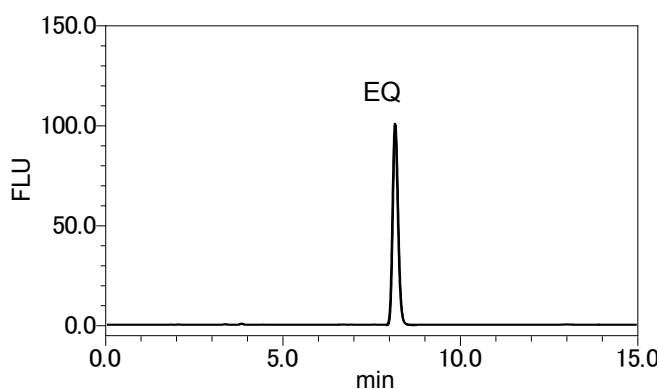


Figure 1 Chromatogram of Standard Solution (20 µg/mL)

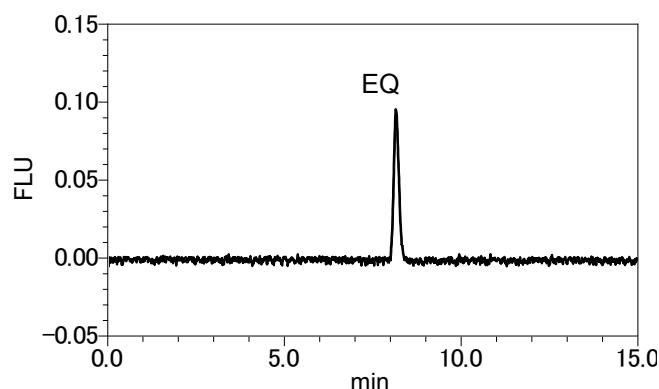


Figure 3 Chromatogram of Standard Solution (0.02 µg/mL)

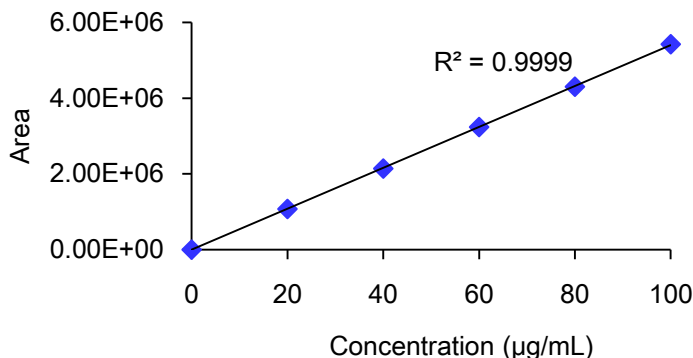


Figure 2 Calibration Curve

Table 1 Analytical Conditions

Column	LaChrom II C18 (5 µm) 4.6 mm I.D.x250 mm
Mobile phase	10 mmol/L CH ₃ COONH ₄ /CH ₃ CN=30/70
Flow rate	1.2 mL/min
Column temperature	25°C
Detector	FL Detector, Excitation wavelength: 360 nm Emission wavelength: 440 nm PMT voltage: Super Low Response: 1 s Sampling period: 200 ms
Injection vol.	5 µL

Table 2 Reproducibility of EQ Standard Solution (0.02 µg/mL) (n=6)

	Retention time (min)	Area
Mean	8.188	1111
%RSD	0.079	0.525

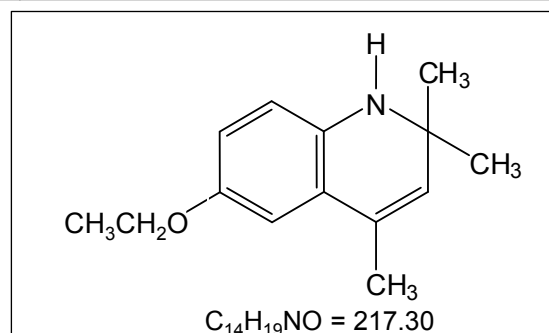


Figure 4 Structural Formula of EQ

- ✓ The calibration curve of EQ (0, 20, 40, 60, 80, 100 µg/mL) showed good linearity with the coefficient of determination of 0.9999 (Figure2).
- ✓ A good result was obtained for the reproducibility (n=6) of the EQ standard solution of 0.02 µg/mL, the quantitation limit for this method (0.0001g/kg) (Table 2).



Preparation Method for Spices

✓ Paprika (powder), black pepper, and white pepper were used as samples.

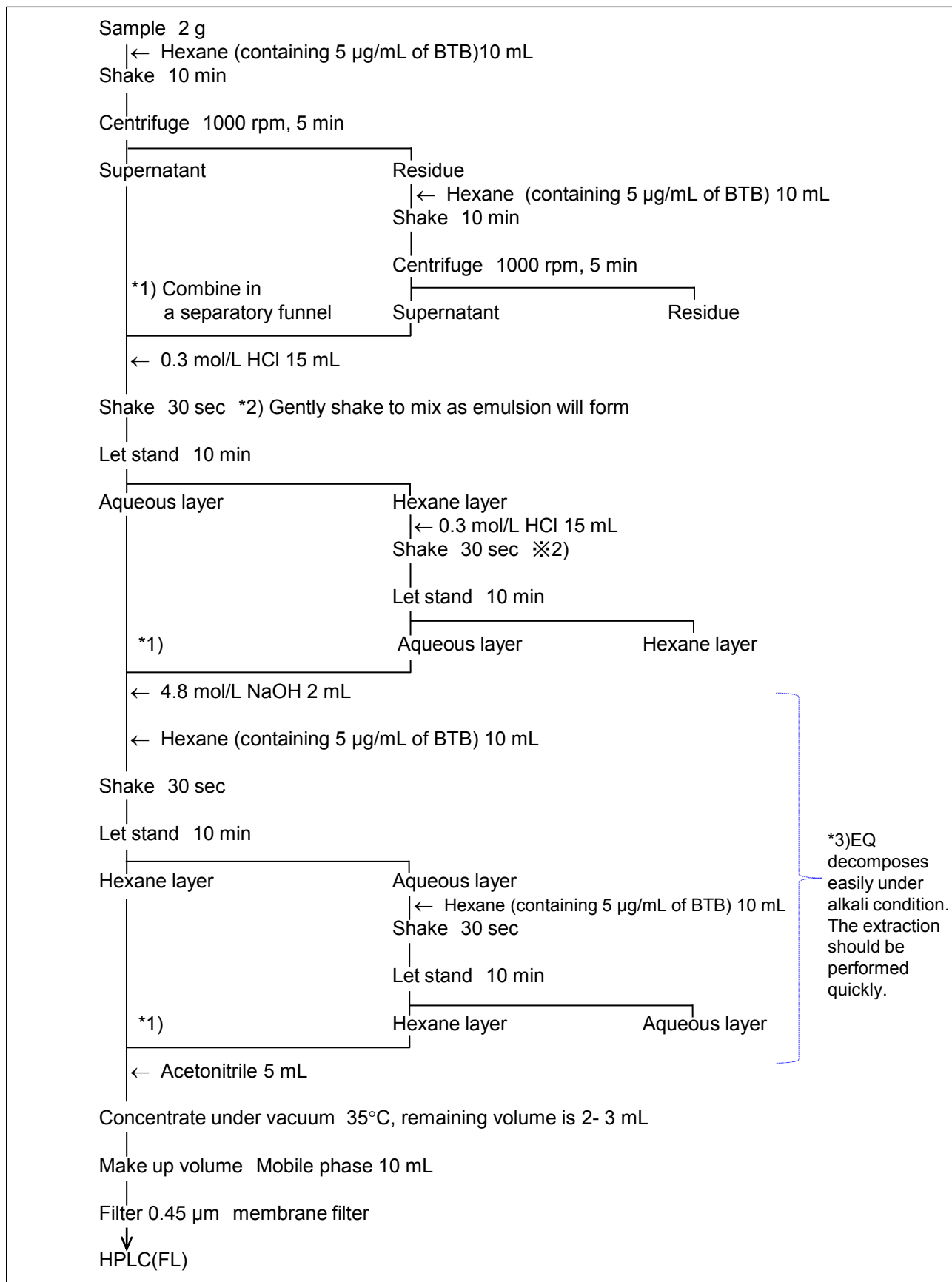


Figure 5 Preparation Method for Spices



Analysis of EQ in Spices

- ✓ The recovery rates determined by adding EQ to the samples at 0.2 µg/mL (0.001 g/kg) were respectively 99.0%, 100%, and 100.5%, for paprika (powder), black pepper, and white pepper, indicating good results (Figure 6, Figure 7, Figure 8, Table 3).
- ✓ No EQ was detected in any of the paprika (powder), black pepper, and white pepper (Figure 6, Figure 7, Figure 8, Table 3).
- ✓ By using Chromaster HPLC system (FL), the selective and accurate analysis of EQ in spices was possible.

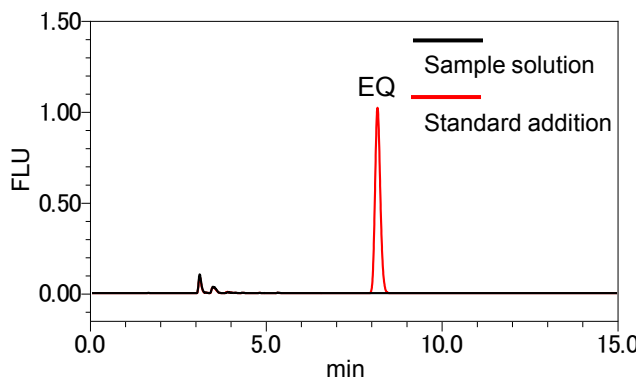


Figure 6 Chromatogram of Paprika (Powder) Sample Solution

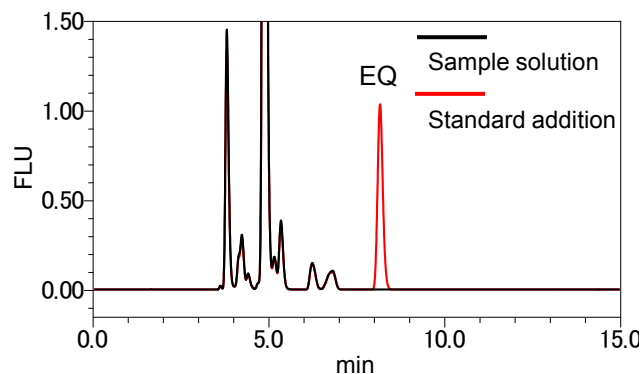


Figure 7 Chromatogram of Black Pepper Sample Solution

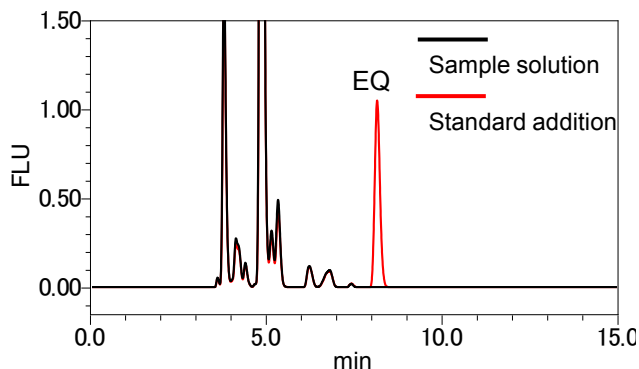


Figure 8 Chromatogram of White Pepper Sample Solution

$$\text{Content (g/kg)} = \frac{\text{Concentration of EQ in Sample Solution (}\mu\text{g/mL)}}{100 \times \text{Amount of Sample (g)}}$$

Table 3 Result of Quantitative Analysis

	Sample solution		Solution with standard addition		
	Sample solution concentration (µg/mL)	Content (g/kg)	Sample solution concentration (µg/mL)	Content (g/kg)	Recovery rate (%)
Paprika (powder) (2.014 g)	n.d.	n.d.	0.198	0.010	99.0
Black pepper (2.023 g)	n.d.	n.d.	0.200	0.010	100
White pepper (2.150 g)	n.d.	n.d.	0.201	0.010	101

<Main system configuration>

Chromaster 5160 Pump, 5260 Autosampler, 5310 Column Oven, 5440 FL Detector

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.