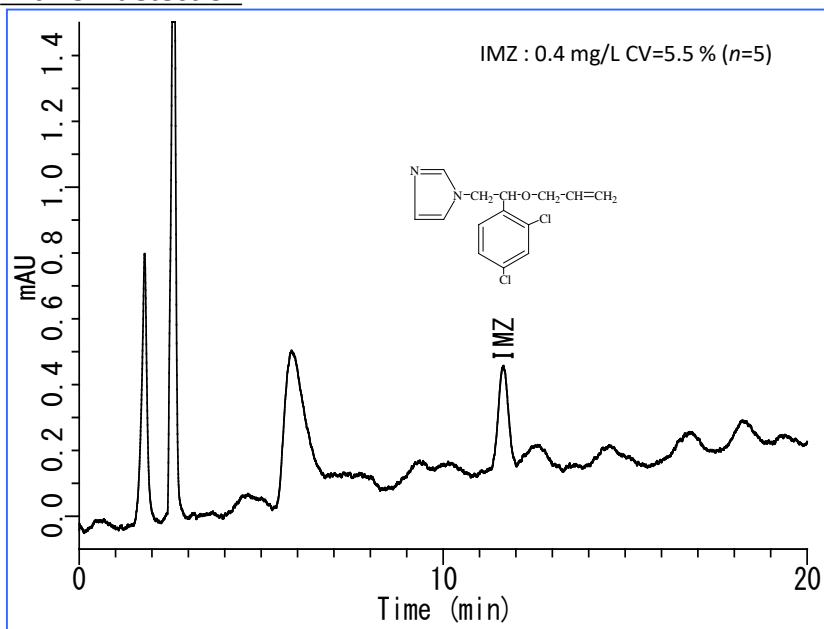


This application note describes the analysis of fungicides in citrus fruits by high-performance liquid chromatography (HPLC) using detection with both fluorescence (FL) and UV absorption (UV).

In the 1980s, fungicides were introduced as post-harvest pesticides and used for long-term storage of fruits and vegetables. Since the 1990s, imported disposable chopsticks have also become a problem. Even today, fungicides are still approved for use in the safety of imported foods, and residue limits have been established.

In order to analyze fungicides, the separate analysis of Imazalil (IMZ) and simultaneous analysis of three other components, orthophenylphenol (OPP), thiabendazole (TBZ) and diphenyl (DP), were carried out under the Hygiene No. 15 Notice Law.

### Example of measurement of an Imazalil standard solution with UV detection

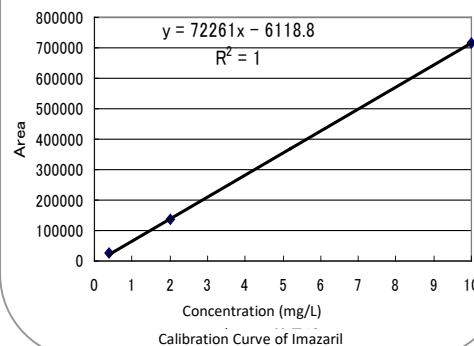


### - Citrus Reference Values

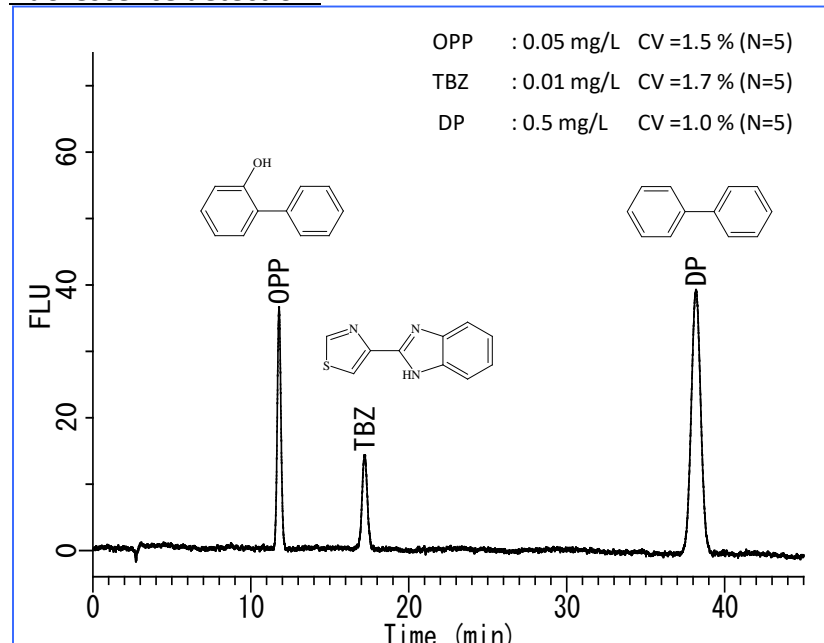
• Imazalil	:5 mg/kg	} Individual analysis
• Orthophenylphenol	:10 mg/kg	
• Thiabendazole	:10 mg/kg	} UV detection
• Diphenyl	:70 mg/kg	
		} Simultaneous analysis

### HPLC conditions

<b>Column</b>	: Inertsil ODS-3 (5 $\mu$ m, 250 x 4.6 mm I.D.)
<b>Eluent</b>	: A) CH <sub>3</sub> OH B) H <sub>2</sub> O A/B = 85/25, v/v (gradient mixer)
<b>Flow rate</b>	: 1.0 mL/min
<b>Column temperature</b>	: 40 °C
<b>Detected</b>	: UV 230 nm
<b>Injection volume</b>	: 10 $\mu$ L

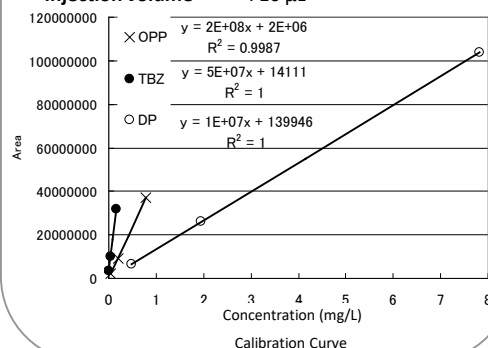


### Example of measurement of orthophenylphenol, thiabendazole, and diphenyl standard solution with fluorescence detection.



### HPLC conditions

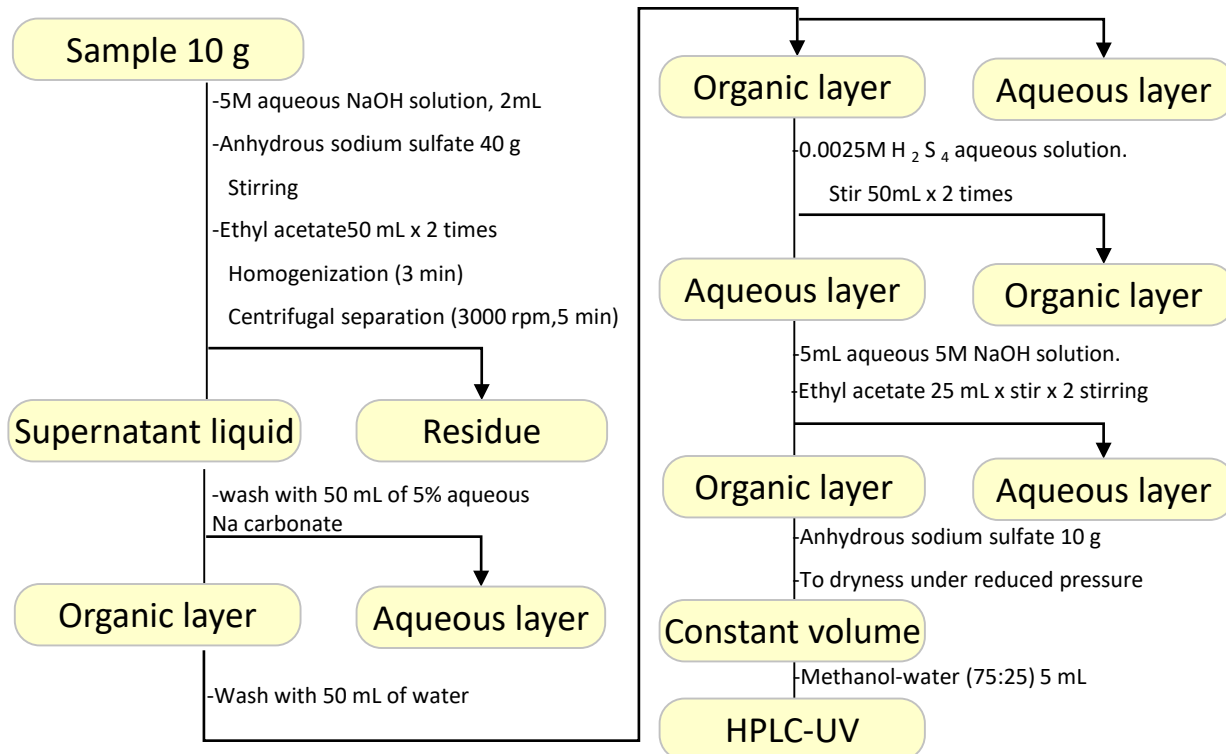
<b>Column</b>	: Inertsil ODS-3 (5 $\mu$ m, 250 x 4.6 mm I.D.)
<b>Eluent</b>	: CH <sub>3</sub> CN : CH <sub>3</sub> OH : H <sub>2</sub> O = 5 : 60 : 35, v/v/v 10 mM SDS added with phosphoric acid at pH 2.5
<b>Flow rate</b>	: 1.0 mL/min
<b>Column temperature</b>	: 40 °C
<b>Detected</b>	: FL Ex. 285 nm Em. 325nm
<b>Injection volume</b>	: 10 $\mu$ L



※Analytical methods for IMZ, OPP, TBZ, and DP in disposable chopsticks under the Food Safety Notification No. 0331002 vary, but the same analytical conditions are used.

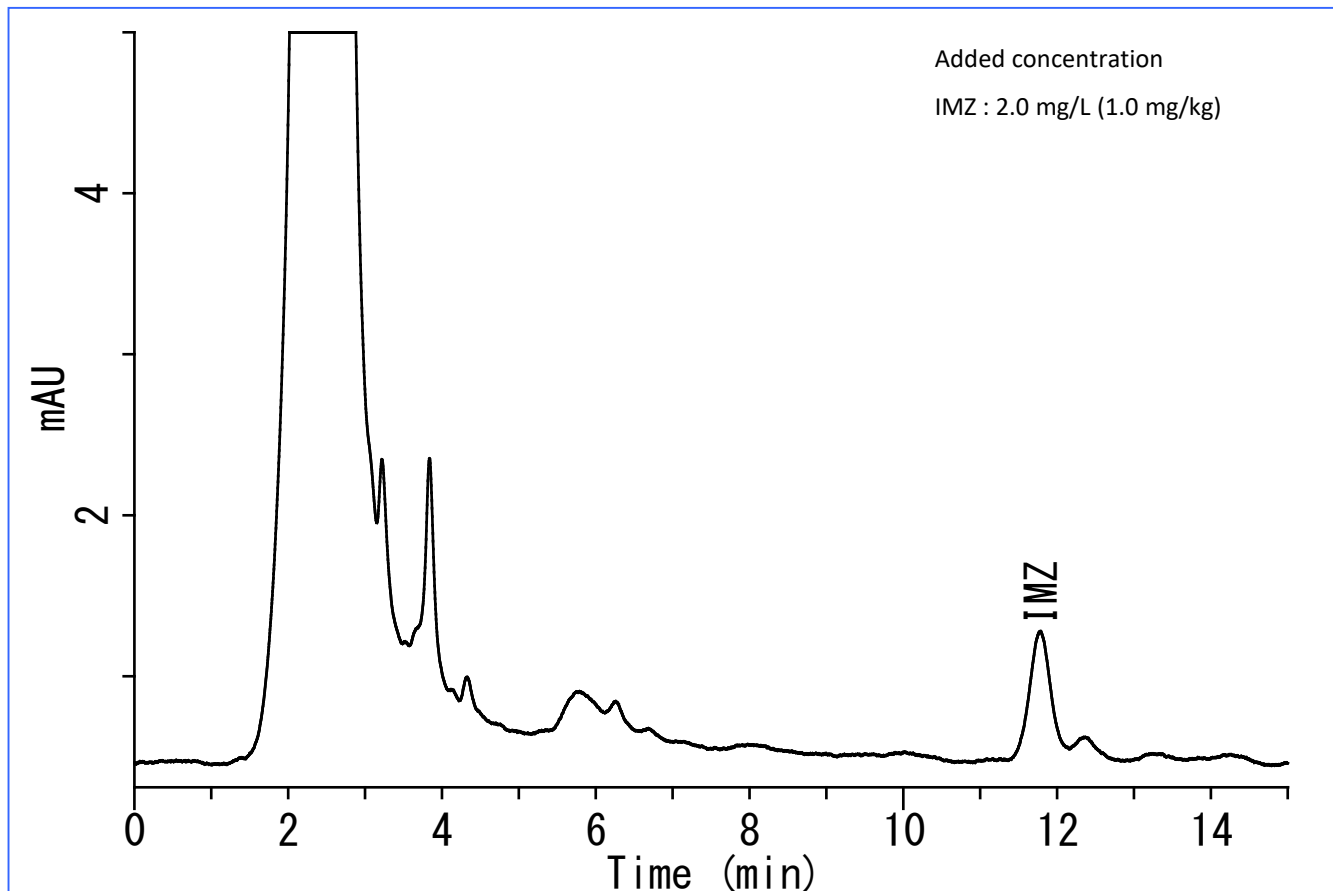
## Imazalyl in lemons

### Pretreatment method :



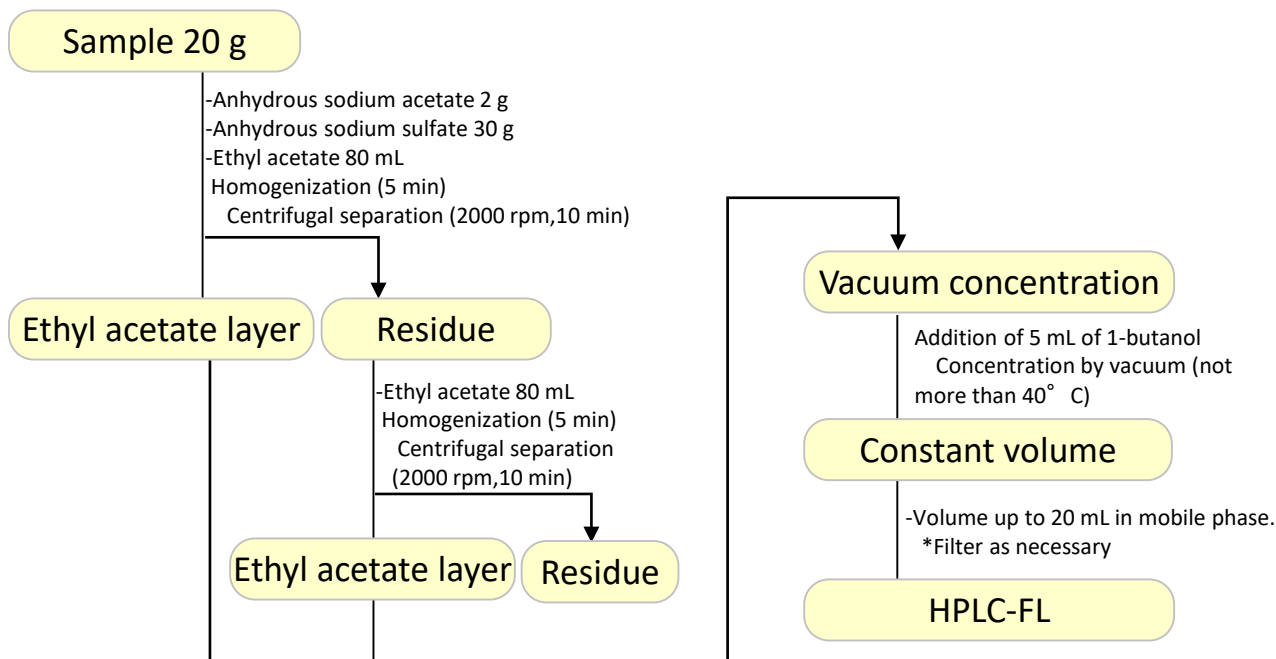
### Chromatogram of standard added sample

Detection is possible at a concentration of  $1/10^{\text{th}}$  or less of the reference value, resulting in a good chromatogram.



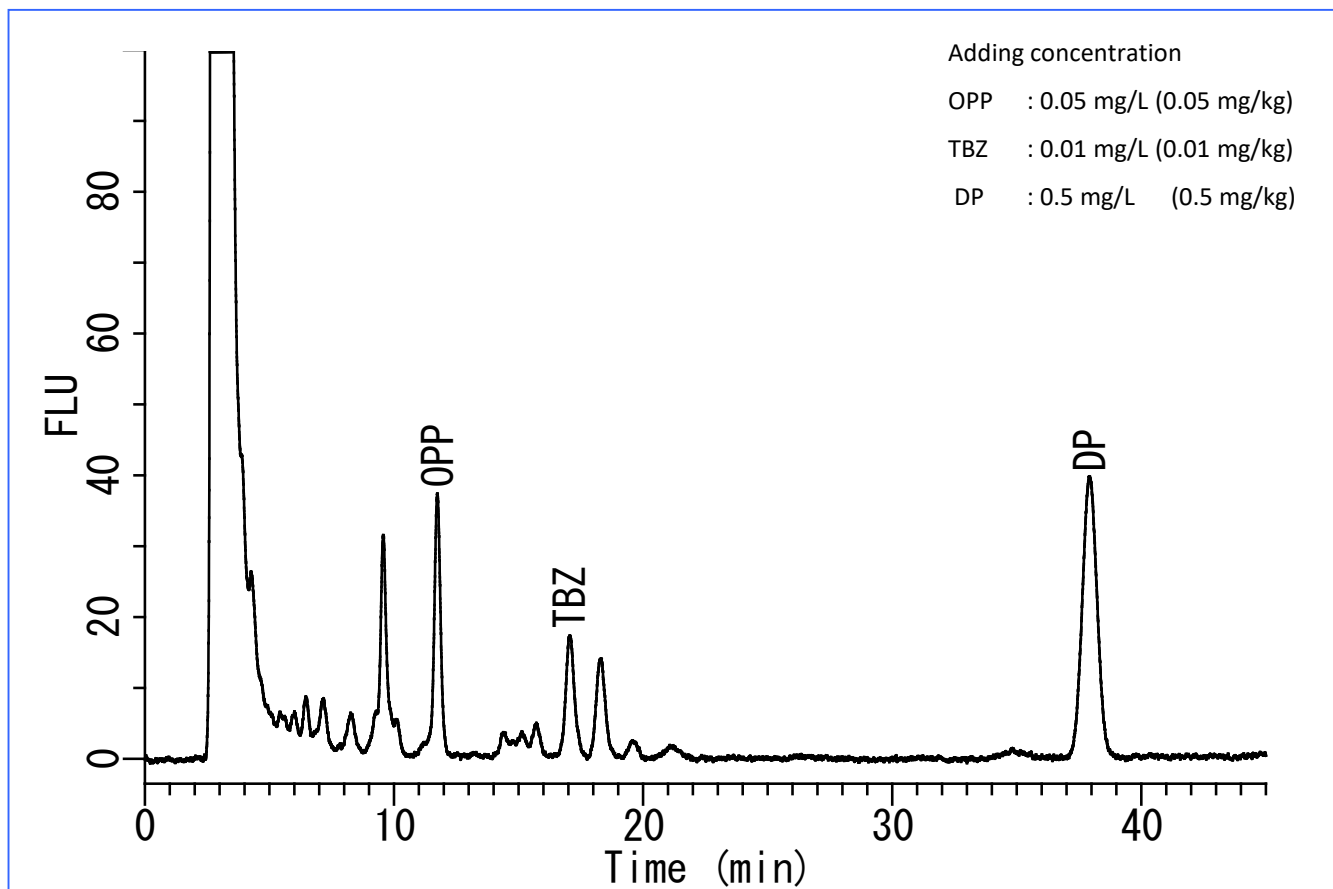
# Orthophenylphenol, thiabendazole, diphenyl in lemon

## Pretreatment method :



## Chromatogram of standard added sample

It is also well separated from contaminants at concentrations of 1/10 th or less of the standard value, and can be quantified.



# IMAZALYL - TEST METHOD FOR ANALYSIS OF PESTICIDE RESIDUES

This section describes an analytical method for imazalil, a residual pesticide in accordance with the Food Safety Notification No. 0124001 of Japan.

## HPLC conditions

**Column** : Inertsil ODS-3  
(5  $\mu$ m, 250 x 4.6 mm I.D.)

**Eluent** : A) CH<sub>3</sub>CN  
B) 10 mM KH<sub>2</sub>PO<sub>4</sub>  
A/B = 70/30, v/v  
(gradient mixer)

**Flow rate** : 0.7 mL/min

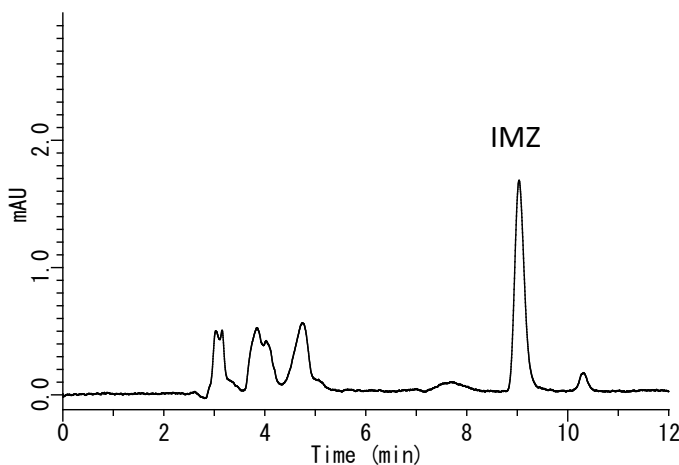
**Column temperature** : 40 °C

**Detected** : UV 230 nm

**Injection volume** : 10  $\mu$ L

## Example: Measurement of standard

IMZ : 5.0 mg/L



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