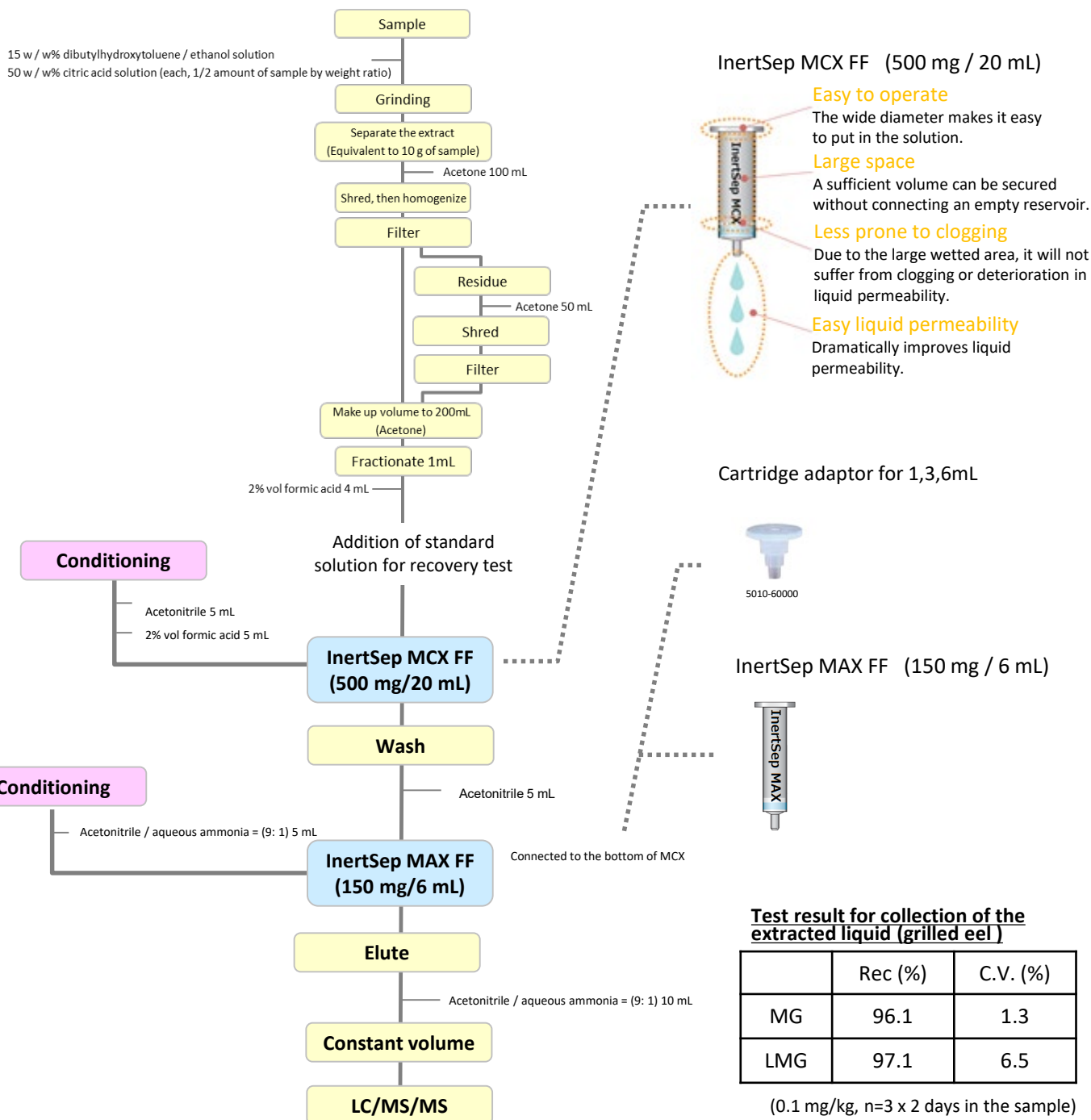


# Analysis of Malachite Green in a Grilled Eel

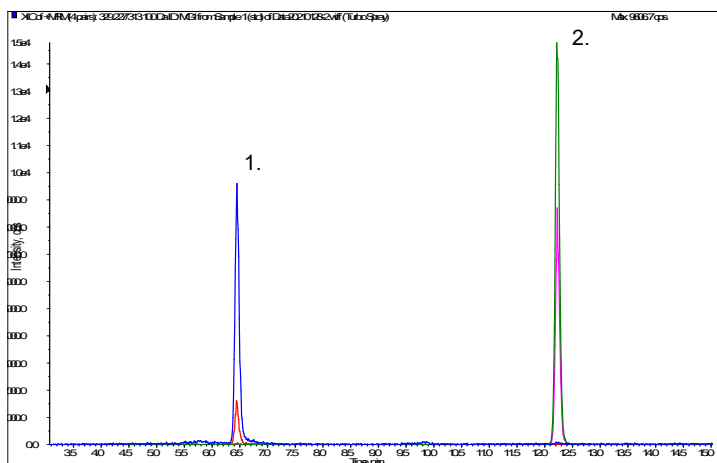
Malachite Green (MG) is a blue-green basic pigment that is used as a remedy against aquatic fungal diseases, it is also used as a bactericidal agent for farmed fish. Current research suggests that it may be carcinogenic, therefore use in foods has been banned in the United States, EU, etc. The distribution and sale of foods in which malachite green is detected is banned in Japan. However, since on rare occasions violations of this ban are confirmed in imported foods, etc., tests are widely conducted by inspection organizations. In this study, malachite green and its biotransform Leucomalachite Green (LMG) were extracted from grilled eel extract and purified using a mixed-mode polymer solid phase (InertSep MCX FF, LMG) and then analyzed by LC / MS / MS with a test method complied with Notification of the Ministry of Health, Labor and Welfare.

## 1. Flow Chart of Solid Phase Pretreatment



## 2. Measurement Conditions

### Example: measurement of standard solution



#### HPLC conditions

**Column** : InertSustainSwift C18  
(5 μm, 150 x 2.1 mm I.D.)

**Eluent** : A) 50 mM HCOONH<sub>4</sub> in H<sub>2</sub>O (pH 3.5)  
B) CH<sub>3</sub>CN

| Time (min) | A% | B% |
|------------|----|----|
| 0.0        | 70 | 30 |
| 15.0       | 10 | 90 |
| 25.0       | 10 | 90 |

**Flow rate** : 0.4 mL/min

**Column** : 40 °C  
temperature

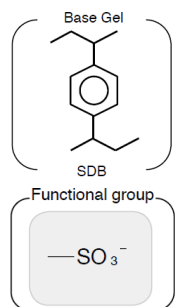
**Detection** : LC-MS/MS  
(4000QTRAP : ESI, Positive, SRM)

**Injection Vol.** : 10 μL

**Sample** : 1. Malachite Green Q1/Q3=329/313 (Quantitation ions)  
329/165 (Reference ions)  
2. Leucomalachite Green Q1/Q3=331/316 (Quantitation ions)  
331/239 (Reference ions)  
(0.5 μg/L each)

## 3. Solid-phase Extraction Column

### [InertSep MCX FF]



Average particle size : 70 μm

Surface area : 480 m<sup>2</sup>/g

Pore volume : 1.1 mL/g

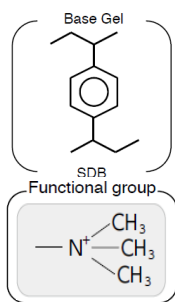
Pore size : 9 nm

pH range : 1~14

InertSep MCX FF is a styrene-divinylbenzene polymer (SDB) solid phase modified with a strong cation exchange group. By exerting both a reverse-phase and cation exchange action, basic compounds can be strongly retained, making it extremely effective for the separation of acidic and neutral contaminants. Since the particle size is as large as 70 μm, it is ideal for biological sample extracts.

| Description     | Column size  | Q'ty /pkg | Cat.No.    |
|-----------------|--------------|-----------|------------|
| InertSep MCX FF | 500 mg/20 mL | 20pcs/pkg | 5010-62704 |

### [InertSep MAX FF]



Average particle size : 70 μm

Surface area : 480 m<sup>2</sup>/g

Pore volume : 1.1 mL/g

Pore size : 9 nm

pH range : 1~14

InertSep MAX FF is a styrene-divinylbenzene polymer (SDB) solid phase modified with a strong anion exchange group. By exerting both a reverse-phase and anion exchange action, acidic compounds can be strongly retained, making it extremely effective for the separation of basic and neutral contaminants. Since the particle size is as large as 70 μm, it is ideal for biological sample extracts.

| Description     | Column size | Q'ty /pkg | Cat.No.    |
|-----------------|-------------|-----------|------------|
| InertSep MAX FF | 150 mg/6 mL | 30pcs/pkg | 5010-62741 |

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