# **Analysis of Cyclamate**

LT086
GL Sciences Inc.

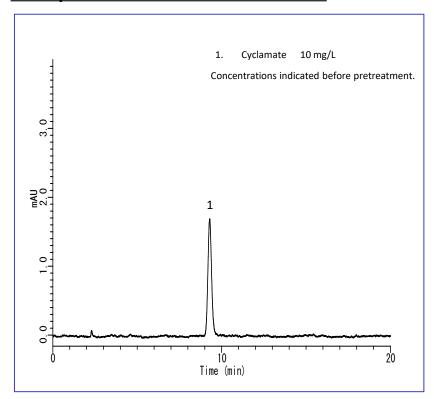
This application describes the analysis of cyclamic acid in foods.

Cyclamic acid (N-cyclohexylsulfamic acid), also called Ticlo, had been used as a sweetener since 1956, but this has been prohibited since 1969, because of reports of suspected carcinogenicity and teratogenicity.

The "test method for cyclamic acid" in the Food Safety Inspection Notification No. 0829009 employs an HPLC method with solid-phase extraction. This report demonstrates that analysis was performed according to this method and excellent data was obtained.

(K.Suzuki, Y.Tanaka)

# **Example: Measurement of standard**



### **HPLC** conditions

Column : Inertsil ODS-3

(5 μm, 150 x 4.6 mm I.D.)

Eluent : A) CH 3 CN

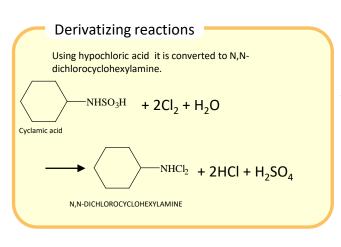
B) H<sub>2</sub>O

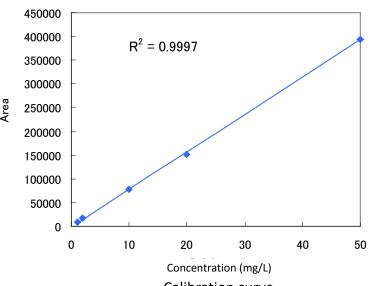
A/B = 70/30, v/v

(Mixed by a gradient mixer)

Flow rate : 1.0 mL/min
Column temperature : 40 °C
Detected : UV 314 nm

Injection volume:  $10 \mu L$ 





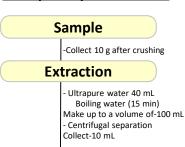
Calibration curve
Concentrations indicated before pretreatment.



# **Example of analysis**

Samples pretreated according to the test method described above were used as measurement samples and analyzed by HPLC.

# **Example of pretreatment**



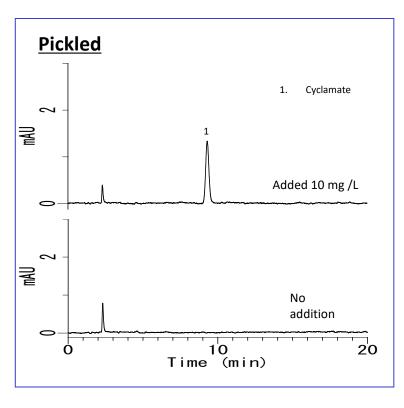
## Solid phase extraction

- Inertsep Slim C18 (900 mg) + Inertsep SAX (500 mg/3 mL)
- Condition each solid phase with 10 mL of methanol and 10 mL of ultrapure water
- 10 mL sample solution
- Wash with 10 mL of purified water
- Transfer 10mL of hydrochloric acid (1 in 100) to Inertsep SAX only, and collect.

#### Derivatized

- To 10 mL of the collected solution add 2 mL of sulfuric acid (1 in 2) and 5 mL of n-hexane,
   Add 1 mL of aqueous sodium hypochlorite
- Shake (1min)
- The hexane layer was separated and used as the sample for measurement.

# **HPLC-UV**



For some samples, the solid-phase cartridge may become clogged. If this occurs, use a solid-phase cartridge with a large fluid drain area, such as Inertsep C18 (1 g/20 mL).

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