

# Certificate of Analysis

**NRC-CNRC**

Certified Reference Material

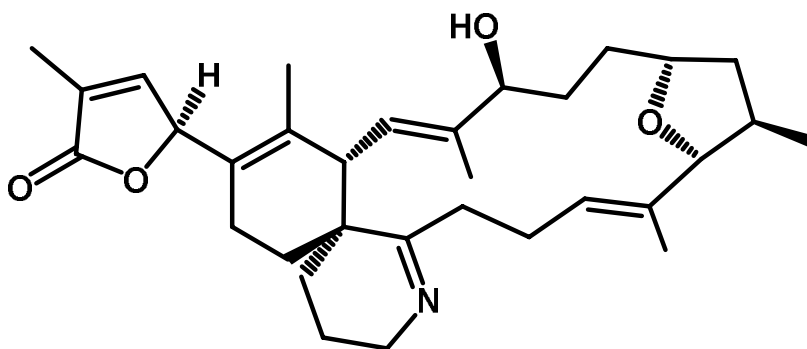
CRM-GYM-b (Lot# 20120712)

Certified Calibration Solution for Gymnodimine

Gymnodimines (GYM) are members of a group of “fast-acting” marine toxins known as cyclic imines [1-5]. Gymnodimine was first observed in shellfish and plankton from New Zealand [1] and has since been observed in various locations [6,7]. Gymnodimine is produced by the dinoflagellate *Karenia selliformis* and causes rapid death in the mouse bioassay [8]. CRM-GYM-b is a certified calibration solution of GYM in methanol containing 0.05% trifluoroacetic acid (TFA) with the concentration reported in Table 1.

**Table 1:** Certified concentration values for CRM-GYM-b.

Compound	$\mu\text{mol/L}$ (at +20 °C)	$\mu\text{g/mL}$ (at +20 °C)	$\mu\text{g/g}$
Gymnodimine	$4.93 \pm 0.26$	$2.50 \pm 0.13$	$3.16 \pm 0.17$



**Gymnodimine**

CAS registry No.: 173792-58-0

Molecular formula:  $\text{C}_{32}\text{H}_{45}\text{NO}_4$

Molecular weight: 507.71

$[\text{M}+\text{H}]^+$ :  $m/z$  508.3421

Expiry date: 1 year from date of sale

Storage conditions: -12 °C or below



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## Intended Use

CRM-GYM-b is a certified calibration solution designed for analytical method development and accurate quantitation of GYM. The concentration of CRM-GYM-b makes it suitable for preparing a dilution series for calibration of liquid chromatography (LC) and liquid chromatography-mass spectrometry (LC-MS) instrumentation, as well as for spiking shellfish control samples for recovery experiments.

## Preparation of CRM-GYM-b

GYM was isolated at the Cawthron Institute (Nelson, New Zealand) from a laboratory culture of *Karenia selliformis*. It was purified through a series of chromatographic steps. The CRM solution was prepared by dissolving pure GYM in degassed, high-purity methanol (containing 0.05% trifluoroacetic acid). This solution was thoroughly mixed with a Teflon stir bar and magnetic mixer while being cooled in an ice bath under an argon atmosphere. Aliquots were dispensed into amber glass ampoules and flame-sealed under argon. Ampoules were then labeled, packaged, and immediately transferred to long-term storage at -80 °C.

## Structural Confirmation and Purity Assessment

The structure and purity of GYM used in the preparation of CRM-GYM-b were confirmed by <sup>1</sup>H nuclear magnetic resonance (NMR) (Figure 1). An LC-MS/MS product ion spectrum showed a fragmentation that is characteristic of GYM (Figure 2).

Purity assessment of the GYM stock solution was carried out with LC-MS. While no major impurities were observed, a number of GYM related impurities ( $[M+H]^+$  at  $m/z$  524.4) were detected at very low levels (<0.1%) (Figure 3). An earlier eluting, unknown GYM isomer comprising <1% of the total GYM concentration was resolved from GYM and detected by LC-MS using different isocratic conditions (Figure 4).

## Homogeneity

A representative number of ampoules ( $n = 24$ ) was selected across the fill series to determine the homogeneity of CRM-GYM-b. Analysis was performed by LC-MS. The between-bottle variation was measured to be smaller than the variation for replicate analyses of one solution (RSD <3% for  $n = 24$ ), thus demonstrating acceptable homogeneity over the entire ampoule range.

## Stability Study

The short-term stability of CRM-GYM-b in methanol (0.05% TFA) was assessed using a reverse isochronous stability study design [9] with -80 °C as the reference temperature. Over a period of 30 days no degradation of GYM was observed at temperatures up to +37 °C, indicating excellent stability over the short-term. Degradation at +37 °C did become significant in a long-term study after 6 months. The uncertainty associated with the long-term stability assessment was determined for inclusion in the final combined uncertainty of CRM-GYM-b using the standard error of the -12 °C slope. The relative uncertainty due to long-term stability ( $\mu_{\text{ITS}}$ ) is 1.14%. The recommended storage temperature for CRM-GYM-b is -12 °C or below.



## Certified Value

The certified value of  $4.93 \pm 0.26$   $\mu\text{mol/L}$  for CRM-GYM-b (Table 1) is based on the results obtained at the National Research Council of Canada (NRC) using two independent analytical methods: quantitative NMR (qNMR) spectroscopy [10] and LC-MS. Calibrations for qNMR were performed using accurately diluted high purity caffeine standards. CRM-GYM (Lot# 20021220) was used for calibration of LC-MS measurements. LC-MS conditions were as follows: Thermo 3  $\mu\text{m}$  Hypersil BDS C8 column (50  $\times$  2.0 mm) maintained at +30 °C; 0.3 mL/min acetonitrile/water (30:70) containing 50 mM formic acid and 2 mM ammonium formate.

The results shown in this certificate are traceable to the International System of Units (SI) standard through gravimetrically prepared standards of established purity. This product serves as a suitable reference material for laboratory quality assurance programs.

## Uncertainty

The overall uncertainty estimate ( $U_{\text{CRM}}$ ) for CRM-GYM-b includes uncertainties associated with batch characterization ( $\mu_{\text{char}}$ ), between-bottle variation ( $\mu_{\text{hom}}$ ), and stability in long-term storage ( $\mu_{\text{stab}}$ ) [11]. These components can be combined and expanded according to:

$$U_{\text{CRM}} = k\sqrt{\mu_{\text{char}}^2 + \mu_{\text{hom}}^2 + \mu_{\text{stab}}^2}$$

where  $k$  is the coverage factor (generally 2).

All reasonable sources of error related to characterization of CRM-GYM-b were considered and quantitated. Quantitative analysis of GYM by qNMR and LC-MS/MS yielded a combined uncertainty ( $\mu_{\text{char}}$ ) value of 0.030  $\mu\text{M}$ . An extensive homogeneity study was performed on CRM-GYM-b and its uncertainty was found to be 0.052  $\mu\text{M}$ . The uncertainty for long-term stability ( $\mu_{\text{stab}}$ ) was established to be 0.056  $\mu\text{M}$ . All these sources of uncertainty were combined with a coverage factor of 2 in the estimate of final uncertainty associated with the certified value of CRM-GYM-b (Table 2).

**Table 2:** Uncertainty budget for the certified value of CRM-GYM-b.

Uncertainties	[ $\mu\text{mol/L}$ ]
$\mu_{\text{hom}}$	0.052
$\mu_{\text{stab}}$	0.056
$\mu_{\text{char}}$	0.030
$U_{\text{CRM}} (k=2)$	0.26

## Storage Instructions

To ensure the stability of CRM-GYM-b, this material should be stored unopened at -12 °C or below. Likewise, any dilutions of this material should be stored in low-headspace vials at -12 °C or below.



## Expiry

If stored unopened at the recommended storage condition of -12 °C or less, the certified concentration of CRM-GYM-b is valid for 1 year from the time of sale.

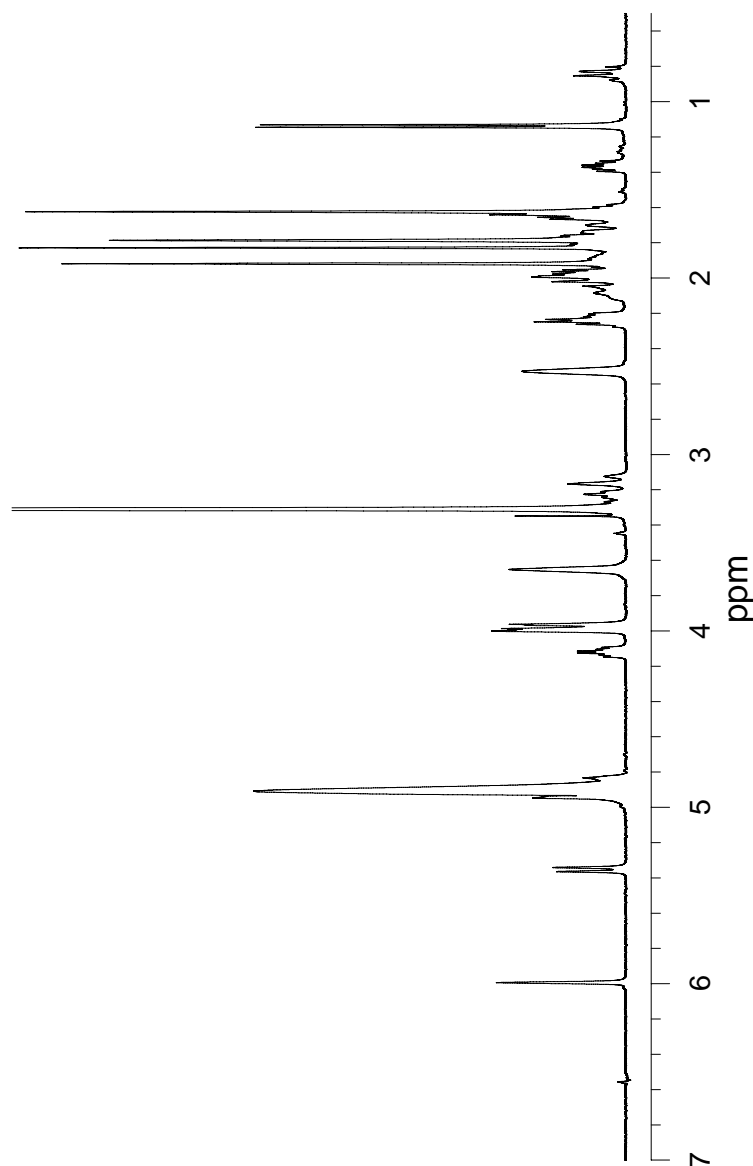
## Instructions for Use

Prior to opening, each ampoule should be allowed to warm to room temperature and the contents mixed thoroughly. The ampoules should be opened at the pre-scored mark. Once an ampoule has been opened, accurate aliquots should be removed with calibrated volumetric equipment and transferred to volumetric flasks or vials. Increases in concentration due to evaporation of methanol will occur if the solution is left opened for more than a few minutes. It is recommended that solutions of CRM-GYM-b should not be evaporated to dryness, due to the potential decomposition on glass surfaces. *Note:* The volume of the solution is not certified. Therefore, the entire contents of the ampoule should not simply be transferred to a volumetric flask and diluted to volume.

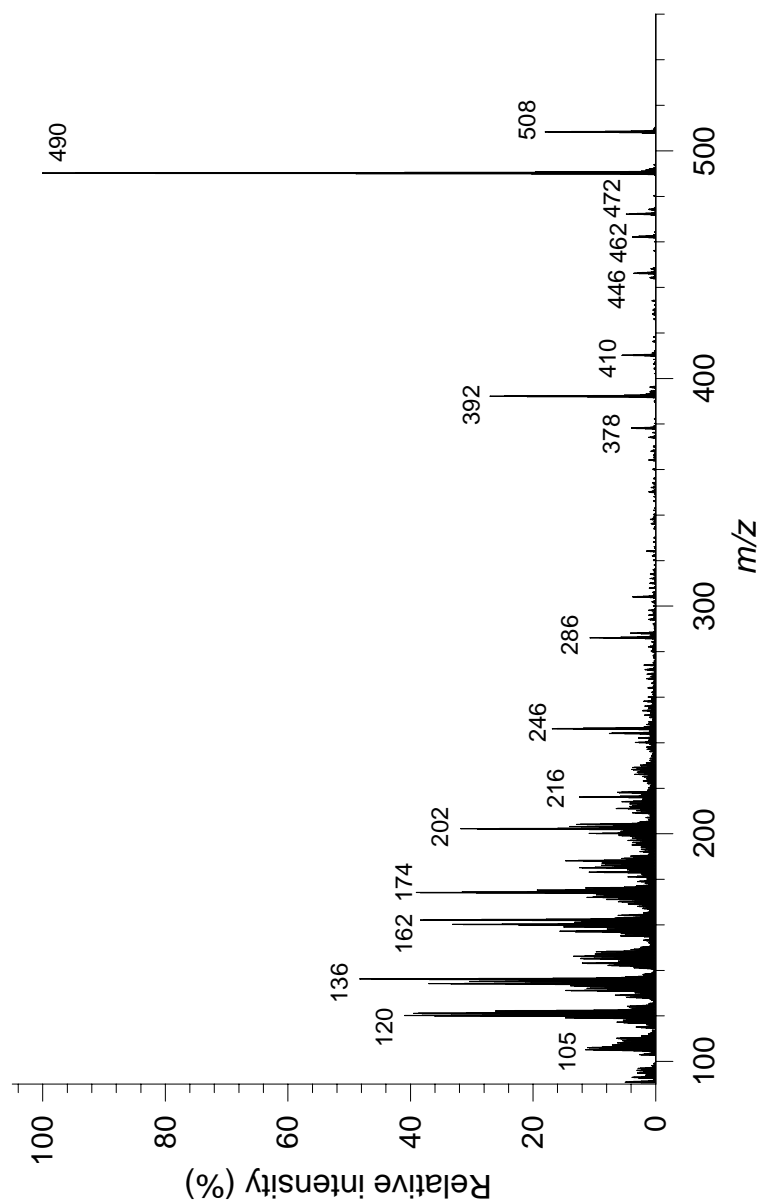
## Safety Instructions

Like the structurally related spirolides, GYM is classified as a fast acting toxin, and studies have demonstrated that GYMs have high toxicity to mice via intraperitoneal injection [8]. No human illness has thus far been reported due to GYM. Nevertheless, precautions should be taken when handling CRM-GYM-b. Inhalation and ingestion of methanol is harmful; ingestion may cause blindness or fatality, while prolonged skin contact may result in dermatitis and/or kidney damage. Only qualified personnel should handle the solution and appropriate disposal methods should be used. Heavy gloves and eye protection should be used when opening the ampoule in the event the glass shatters. A material safety data sheet (MSDS) is available for CRM-GYM-b.

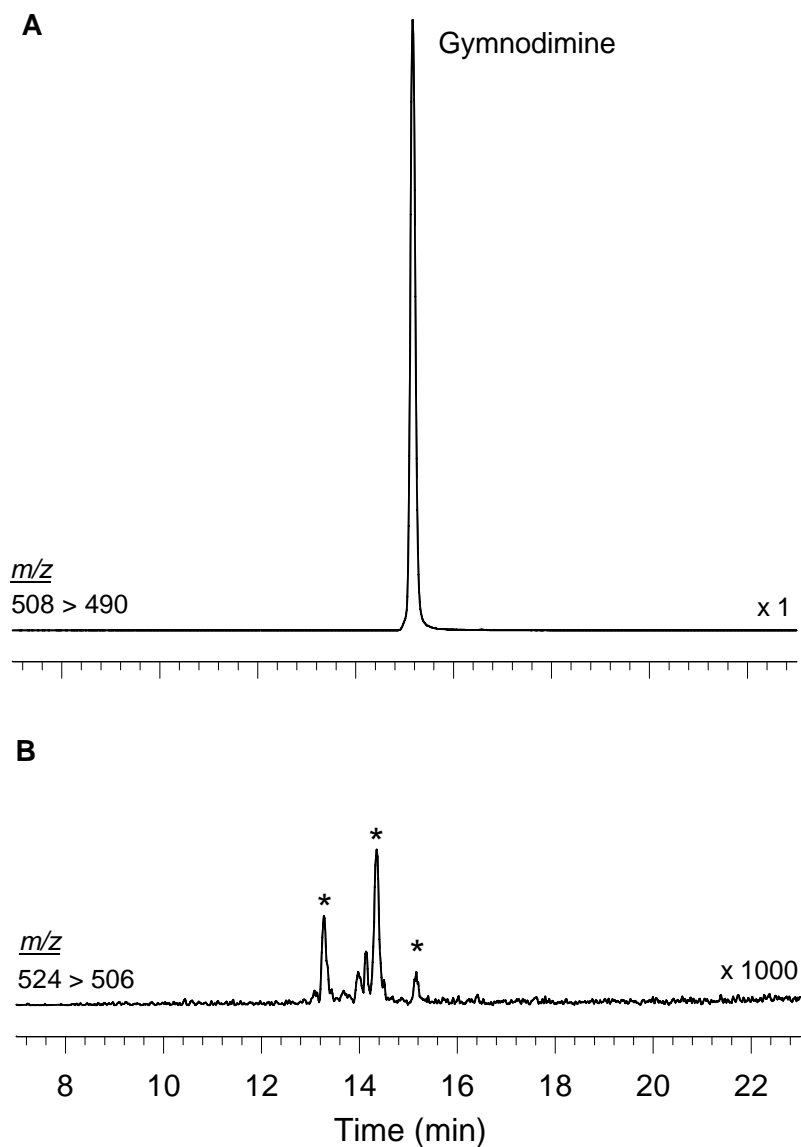


**Figures**

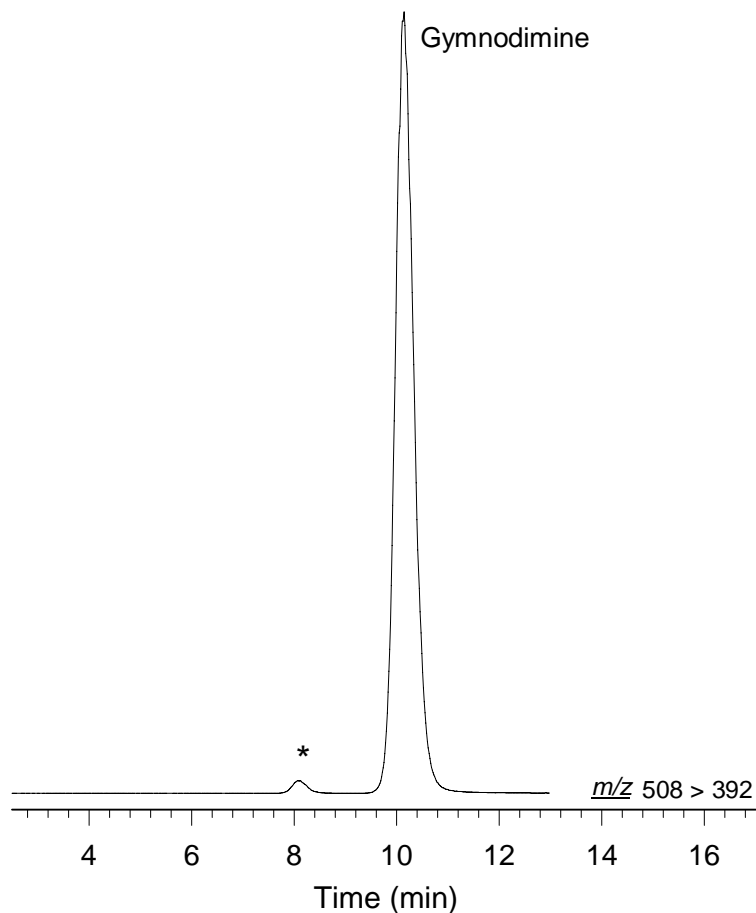
**Figure 1:**  $^1\text{H}$  NMR spectrum of GYM dissolved in methanol- $\text{d}_3$  (99.5% D, Cambridge Isotope Laboratories).



**Figure 2:** Tandem MS product ion spectrum of the  $[M+H]^+$  ion ( $m/z$  508) of GYM acquired on an AB SCIEX 5500 QTRAP mass spectrometer using a collision energy of 55 eV.



**Figure 3:** LC-MS analysis of CRM-GYM-b and detection of trace level impurities ( $m/z$  524>506, marked with asterisks). Conditions: Thermo 3  $\mu$ m Hypersil C8 column (150  $\times$  2 mm) maintained at +20  $^{\circ}$ C; water (A) and acetonitrile/water (95/5) (B) both containing 50 mM formic acid and 2 mM ammonium formate with gradient elution from 5 to 100% B over 25 min at 0.2 mL/min; AB SCIEX 5500 QTRAP mass spectrometer operated in positive selected reaction monitoring mode.



**Figure 4:** LC-MS/MS analysis of CRM-GYM-b stock solution showing an unknown GYM isomer resolved under isocratic conditions (isomer marked with asterisk). Conditions: Phenomenex 3  $\mu$ m Luna C8 column (50  $\times$  2.1 mm) maintained at +20 °C and eluting at 0.2 mL/min with methanol/water (25:75) containing 50 mM formic acid. The isomer comprised ~1% when monitored in selected ion monitoring mode.



## References

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**Signed :** 

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**This Certificate is only valid if the corresponding product was obtained directly from NRC or one of our qualified vendors.**

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