Analytical information

Name: Azaspiracid-4 in methanol

Code: 02-AZA4 Batch: 20-001

General description:

Product Specification	
MOLECULAR FORMULA:	$C_{46}H_{69}NO_{13}$
MOLECULAR WEIGHT:	844.039
MONOISOTOPIC MW	843.477
CAS NUMBER:	344422-49-7
CONCENTRATION:	(1.31 ± 0.03) µg/mL †
PURITY:	≥ 98% ‡
FORMULATION:	Liquid. In methanol.
SHIPPING:	SHIPPED ON ICE
LONG TERM STORAGE:	-20°C
HAZARD:	See MSDS.

† **20-001 batch concentration of Azaspiracid-4 was settled by UPLC MS/MS versus Azaspiracid-1 calibration curve where the assumption of an equal response factor for toxin quantification was performed.**

‡ Azaspiracid-4 was purified via preparative HPLC-MS/MS from mussel matrix. Analytical UPLC-MS/MS were used to quantify and check purity.

Instructions for proper handling use: This material contains toxic material, and should be handled with care. Read MSDS before using. Material Safety Data sheet for information regarding 02-AZA4 is enclosed in the package. Use proper disposal methods.

Before opening an ampoule it must be guaranteed that the content is a liquid and properly mixed to ensure homogeneity. Sample aliquots for analysis should be withdrawn at 15 °C to 25 °C immediately after opening the ampoules and should be processed without delay for the concentration value to be valid within the stated uncertainty. Ampoule should not remain open longer than few minutes due to solvent volatility and air contamination.

Instructions for appropriate conditions of storage: The original vial should be stored at temperatures below/at -20 ºC (freezer) until use. Unopened ampoules should be stored upright under normal laboratory conditions inside the original container supplied.

Property values:

Date of analysis:	**June, 08th 2020**
Concentration of Azaspiracid-4 in methanol solution	**(1.31 ± 0.03) µg / mL**

Estimated uncertainty corresponds to a level of confidence in terms of one standard deviation.

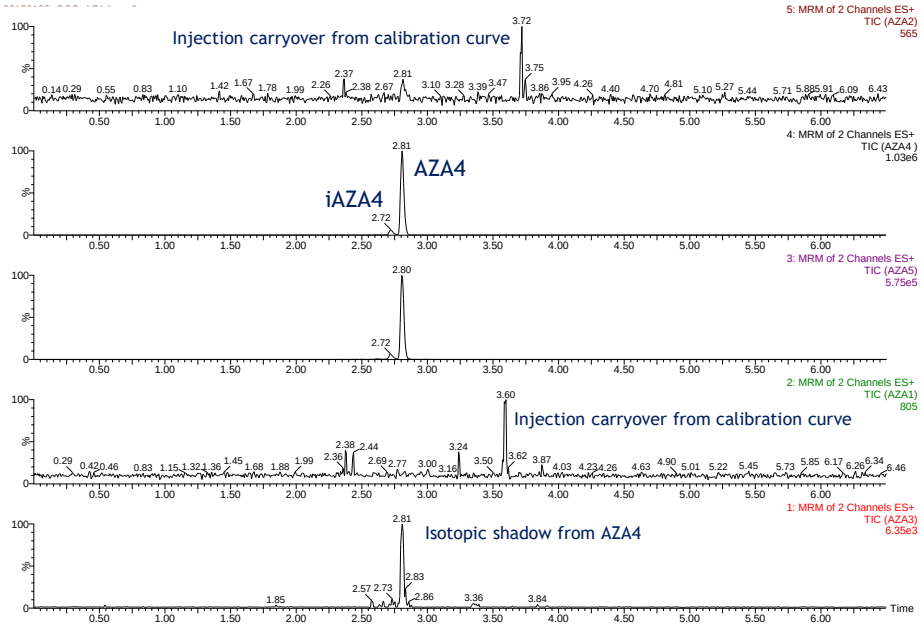

Figure 1. Analysis of the **02-AZA4** solution by UPLC-MS/MS. Conditions: ACQUITY UPLC BEH C18 1.7µm, 100x2.1 mm, UPLC column. Elution conditions, gradient mode with mobile phases: (A) Water and (B) Aqueous 95% acetonitrile, both with 2mM ammonium formate and 50mM formic acid; Gradient: 0.00 min 30% B; 3.00 min 90% B; 4.50 min 90% B; 4.51 min 30% B; 6.50 min 30% B; flow rate: 0.40 mL/min. Temperature: 30 °C. MS detector: Waters XEVO TQ using the typical MRM transitions of Azaspiracid family. Isomer of Azaspiracid-4 is the major impurity (1.2 %). Traces of AZA2 and AZA1 were detected but most of these signals are due to a process of injection carryover from calibration curves.

Inquiries concerning this material should be directed to:

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This Analytical Information is only valid if the product was obtained directly from Laboratorio CIFGA S.A. or one of our authorized distributors.

Certificate Revision History: *June, 08th 2020* (Original certificate issue date, revision 1).