

Certified Reference Material

Reference material Certificate

Spinetoram solution

Product no.: 43065
Lot no.: BCKK5877
Description of CRM: Pesticides Standard, mixture of spinetoram J and spinetoram L in Acetonitrile
Expiry date: JUN 2025
Storage: Store at -20°C ± 5°C
Molecular formula
 $C_{42}H_{69}NO_{10}$ Spinetoram J
 $C_{43}H_{69}NO_{10}$ Spinetoram L
Molecular mass:
748.01 g/mol Spinetoram J
760.02 g/mol Spinetoram L
CAS No.: 935545-74-7
Density at 20°C: 783.7 kg m⁻³ ± 0.4 kg m⁻³ (k=2)

TraceCERT®
Traceable Certified Reference Materials

Constituent	Certified values at 20°C and expanded uncertainties, $U = k \cdot u$ ($k = 2$) ^{[1][2]}	
Spinetoram (sum of J & L)	126.4 mg/kg ± 13.9 mg/kg	99.1 mg/L ± 10.9 mg/L
Spinetoram J	97.1 mg/kg ± 12.6 mg/kg	76.1 mg/L ± 9.8 mg/L
Spinetoram L	29.3 mg/kg ± 4.4 mg/kg	23.0 mg/L ± 3.4 mg/L

Metrological traceability: Traceable to NIST SRM. Details see "Certification process details".^[3]
Measurement method: The certified value is established by weighing of well characterized starting materials in accordance with ISO/IEC 17025^[4]
Intended use: Use this certified reference material (CRM) as a calibrant for pesticide testing in cannabis or any other appropriate matrix.
Instructions for handling and correct use: Shake well and allow to warm to room temperature before opening the ampule. Open a new ampule for each measurement series.
Health and safety information: Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.
Packaging: Amber glass ampule
Accreditation: Sigma-Aldrich Production GmbH is accredited by the Swiss accreditation authority SAS as registered reference material producer SRMS 0001 in accordance with ISO 17034^[5].
Certificate issue date: June 28, 2023



ISO 17034
SRMS 0001

H. Sprecher – CRM Operations

Dr. P. Zell – Approving Officer



Certification process details:

To guarantee top reliability of the values for this *TraceCERT*[®] certified reference material, well-established procedures were followed. The values have to agree in the range of their uncertainties, but the value from the gravimetric preparation has been chosen as certified value:

1. Gravimetric preparation using well-characterized materials is a practical realization of concentration units, through conversion of mass to amount of substance ^[6]. If the purity of the materials is demonstrated and if contamination and loss of material is strictly prevented this approach allows highest accuracy. The certified value of *TraceCERT*[®] reference materials is based on this approach and directly traceable to the SI unit kilogram. Therefore, comprehensively characterized materials are used. All balances are certified by DKD and calibrated with OIML Class E2 (up to 12 kg) and F2 (up to 64 kg) weights.
2. The starting material is measured against a certified reference material which is traceable to SRM[®] from NIST followed by gravimetric preparation using balances calibrated with SI-traceable weights. Consequently, the value calculated by this unbroken chain of comparisons is traceable to the reference to which the starting material is compared. Due to the nature of qNMR measurements, different analytes can be characterized using the same SRM[®] from NIST (see table „Details on starting material“ on page 3).
3. The mix in its final packaged form is measured by LC to underpin the gravimetric values.
4. Density measurement is performed in accordance with ISO/IEC 17025^[4]
5. The certificate is designed in accordance with ISO Guide 31. ^[7]

Homogeneity assessment:

Due to the nature of the production process, a homogeneous solution derives. Nevertheless, homogeneity is assessed by LC measurements and a homogeneity contribution is included into the calculation of content uncertainty of this CRM.

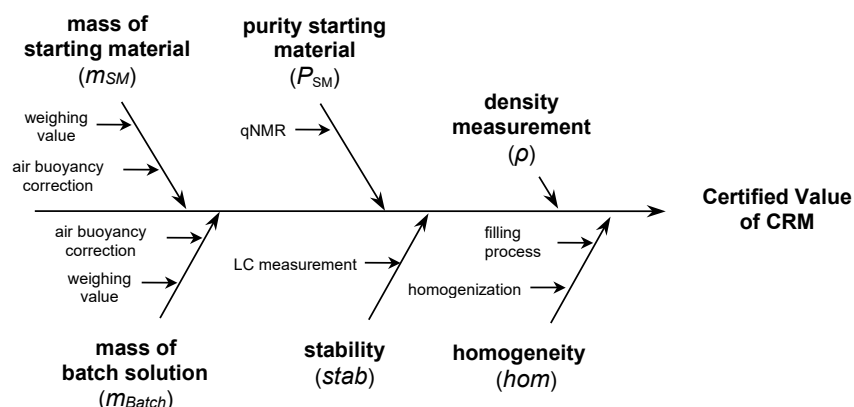
Stability assessment:

A stability study is performed with samples which are stored at different temperatures. The material is tested by LC-MS after certain time intervals and a stability component is included in the overall uncertainty.

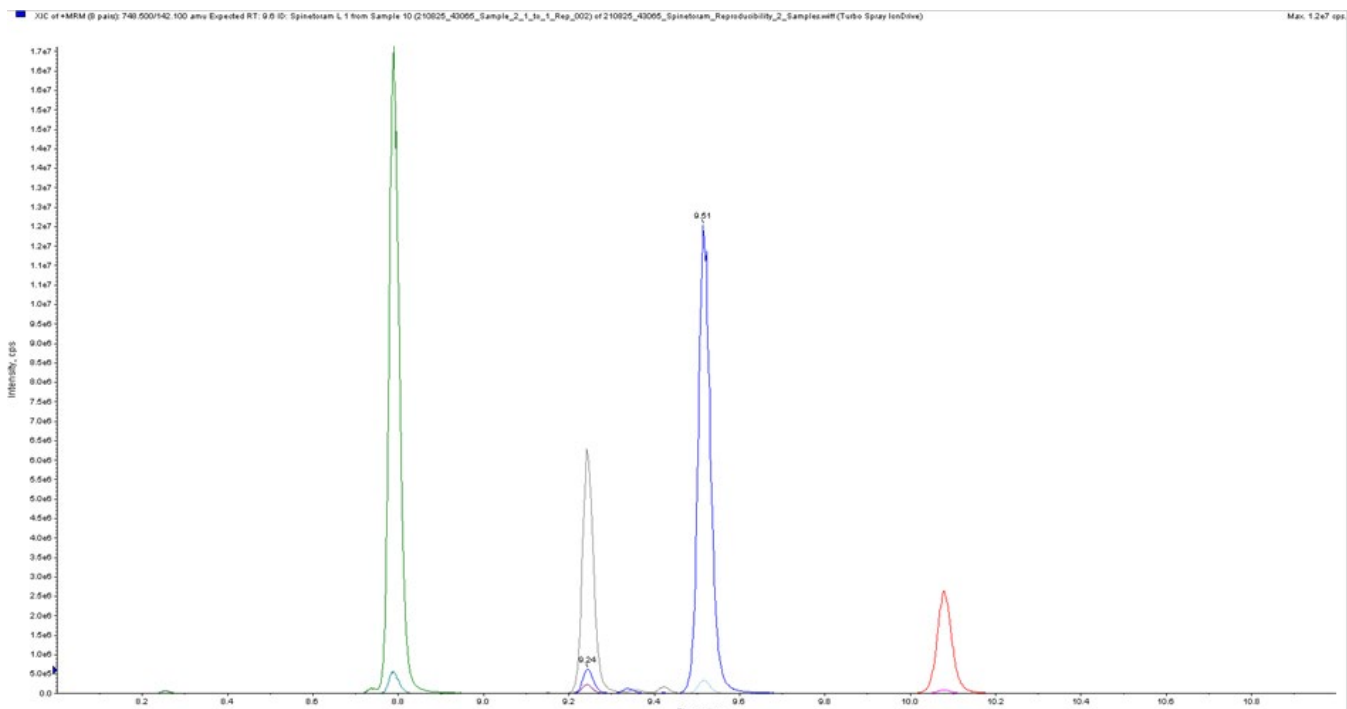
All sample and reference solutions are mixed with an internal standard solution according to "Guidelines for achieving high accuracy in IDMS" ^[8] to give matching signal ratios. When available isotopologues of the corresponding compound are used, for all other compounds closely related isotopologues of the given mixture serve as internal standard. The mixtures are prepared gravimetrically. The analysis of the sample is bracketed by the reference and repeated several times to minimize instrumental variations. The repeatability is included in the combined measurement uncertainty. The two isomers are analyzed by one LC-MS method. The method was optimized and validated for this isomer mixture.

Uncertainty evaluation:

The uncertainty contributions are illustrated by the following cause-effect diagram:



The combined standard uncertainty is calculated by combination of the standard uncertainties of the input estimates according to Eurachem/CITAC Guide "Quantifying Uncertainty in Analytical Measurement" and ISO 17034. ^{[2][3]} Expanded uncertainty is then calculated to a confidence level of 95%, typically by multiplying with a coverage factor of $k=2$.



Shimadzu Nexera / Sciex 6500 TQD

Columns: Ascentis Express C18 (100 x 2.1 mm, 2.0 µm)

Temperature: 50 °C

Eluent A: 0.1 % formic acid / water

Eluent B: 0.1 % formic acid / acetonitrile

Gradient:	time / min	A / %	B / %	flow / mL/min
	0.5	10	90	0.6
	7.1	50	50	
	10.0	50	50	
	10.5	98	2	
	15	98	2	
Reconstitute:	5.0	10	90	0.6

Detection: ESI (+), MRM (Sciex 6500 TQD)

Ion Spray Voltage: 5000 V (von 8 Minuten bis 11 Minuten)

Heater: 500 °C

Ion Source Gas 1: 55 psi (nitrogen)

Ion Source Gas 2: 50 psi (nitrogen)

Curtain Gas: 40 psi (nitrogen)

Collision Gas (CAD): 8 psi (nitrogen)

Inj. vol.: 1 µl

Peak Table:

Compound	Q1 Mass (Da)	Q3 Mass (Da)	RT(min)	DP (volts)	EP (volts)	CE (volts)	CXP (volts)
Spinetoram L 1	748.5	142.1	9.5	80	10	40	5
Spinetoram J 1	760.6	142.1	10.1	30	10	40	15
Spinosyn A 1*	732.6	142.1	8.8	100	10	30	15
Spinosyn D 1*	746.6	142.1	9.2	100	10	40	15
Spinetoram L 2	748.5	203.2	9.5	70	10	40	10
Spinetoram J 2	760.6	203.2	10.1	30	10	40	15
Spinosyn A 2*	732.6	189.1	8.8	100	10	40	10
Spinosyn D 2*	746.6	189.1	9.2	90	10	40	5

*Spinosad (mixture of Spinosyn A and Spinosyn D) was used as internal standard in the HPLC-MS experiment

Details on starting materials:

Compound	CAS Number	Method	NIST SRM traceability
Spinetoram	935545-74-7	qNMR	NIST PS1 (Benzoic Acid)

References:

- [1] ISO Guide 35:2017, "Reference materials - Guidance for characterization and assessment of homogeneity and stability"
- [2] Eurachem/CITAC Guide, 3rd Ed. (2012), "Quantifying uncertainty in analytical measurement"
- [3] Eurachem/CITAC Guide, 2nd Ed. (2019), "Metrological traceability in chemical measurement" ISO
- [4] The accredited testing laboratory STS 0490 performs the measurements and weighing steps for the certification of this CRM under ISO/IEC 17025:2017, "General requirements for the competence of testing and calibration laboratories"
- [5] 17034:2016, "General requirements for the competence of reference material producers"
- [6] Reichmuth, A., Wunderli, S., Weber, M., Meyer, V. R. (2004), "The uncertainty of weighing data obtained with electronic analytical balances", Microchimica Acta 148: 133-141.
- [7] ISO Guide 31:2015, "Reference materials - Contents of certificates, labels and accompanying documentation"
- [8] Sargent, M., Harrington, C., Harte, R. (eds.), "Guidelines for achieving high accuracy in IDMS", LGC (RSC), Cambridge, 2002.

Certificate of analysis revision history:

Certificate version	Date	Reason for version
01	June 28, 2023	Initial version

