RAPPORTI ISTISAN 22|29

ISSN: 1123-3117 (cartaceo) • 2384-8936 (online)

Results of the proficiency test on plant protection products in 2022

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AMBIENTE E SALUTE

ISTITUTO SUPERIORE DI SANITÀ

Results of the proficiency test on plant protection products in 2022

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Rapporti ISTISAN 22/29

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Results of the proficiency test on plant protection products in 2022.
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2022, v, 36 p. Rapporti ISTISAN 22/29

In 2022, the fifth Proficiency Test (PT) on plant protection products available on the Italian market was organized. The aim was to find out the quantity of active ingredient on the different formulation of the plant protection products. Eight Italian laboratories and seventeen worldwide laboratories, who routinely deal with pesticides, were invited to participate. Participation is voluntary and four Italian and sixteen European laboratories have joined. All laboratories obtained data with acceptable values of z-score within the limits $-3.5 \leq Z \leq +3.5$.
Key words: Proficiency test; Plant protection products; Bentazone; Spinosad; Tau Fluvalinate

Istituto Superiore di Sanità
Risultati dell'esercizio interlaboratorio sui prodotti fitosanitari nel 2022.
Angela Santilio, Roberto Cammarata, Valentina Picardo
2022, v, 36 p. Rapporti ISTISAN 22/29 (in inglese)

Nel 2022 è stato organizzato il quinto esercizio interlaboratorio su prodotti fitosanitari disponibili sul mercato nazionale. L'esercizio riguardava la determinazione del contenuto di principio attivo presente in prodotti fitosanitari di diversa formulazione. Sono stati invitati a partecipare 8 laboratori italiani preposti al controllo dei prodotti fitosanitari e 17 laboratori mondiali interessati ai controlli sui prodotti fitosanitari. La partecipazione è su base volontaria e hanno aderito quattro laboratori italiani e sedici europei. Tutti i laboratori hanno ottenuto risultati con valori di *z-score* entro i limiti definiti $-3{,}5 \leq Z \leq +3{,}5$.
Parole chiave: Esercizio interlaboratorio; Prodotti fitosanitari; Bentazone; Spinosad; Tau Fluvalinate

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Citare questo documento come segue:
Santilio A, Cammarata R, Picardo V. *Results of the proficiency test on plant protection products in 2022.* Roma: Istituto Superiore di Sanità; 2022. (Rapporti ISTISAN 22/29).

Legale rappresentante dell'Istituto Superiore di Sanità: *Silvio Brusaferro*
Registro della Stampa - Tribunale di Roma n. 114 (cartaceo) e n. 115 (online) del 16 maggio 2014

Direttore responsabile della serie: *Paola De Castro*
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La responsabilità dei dati scientifici e tecnici è dei singoli autori.

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TABLE OF CONTENTS

ABBREVIATIONS

AAPCO	Association of American Pesticide Control Officials
AFSCA	Agence fédérale pour la sécurité de la chaîne alimentaire (Federal Agency for the Safety of the Food Chain)
CAS	Chemical Abstract Service
CIPAC	Collaborative International Pesticide Analytical Council
CS	Capsule Suspension
CV	Coefficient of Variation
DAD	Diode Array Detector
FID	Flame Ionisation Detector
GC	Gas Chromatography
HRMS	High Resolution Mass Spectrometry
HPLC	High Performance Liquid Chromatography
ISO	International Organization for Standardization
ITPT	Italian Proficiency Test
LC	Liquid Chromatography
MAD	Median absolute deviation
MS	Mass Spectrometry
N/A	Not Available
PDA	PhotoDiode Array
PTFE	Polytetrafluoroethylene
PPP	Plant Protection Product
PPP01	Plant Protection Product number 1
PPP02	Plant Protection Product number 2
PPP03	Plant Protection Product number 3
PRSD	Predicted Relative Standard Deviation
PT	Proficiency Test
RSD	Relative Standard Deviation
SC	Suspension Concentrate
SD	Standard Deviation
SL	Soluble Concentrate
UV	UltraViolet
VIS	Visible
VWD	Variable Wavelength Detector
z-score	Standard Score

Symbols

σ_{PT}	standard deviation for proficiency test
T-test	statistic test of Student's t distribution

PREFACE

The European legislation on Plant Protection Products (PPPs) – Regulation (EC) 1107/2009 – regulates the authorisation, placing on the market, use and control of PPPs and of any active substances, safeners, synergists, co-formulants and adjuvants, which they might contain or which they might consist of.

The objective of those rules is to ensure a high level of protection of both human and animal health and of the environment through evaluation of the risks posed by PPPs, while improving the functioning of the Union market through harmonisation of the rules for their placing on the market and improving agricultural production.

In addition, the Regulation (EU) 2017/625 establish a harmonised European Union framework for the organisation of official controls and official activities taking into account the rules on official controls laid down in Regulation (EC) 882/2004 and in relevant sectoral legislation, and the experience gained from the application of those rules.

The laboratories designated by the competent authorities to perform analyses on PPP samples taken in the context of official controls should possess the expertise, equipment, infrastructure and staff to carry out such tasks to the highest standards. To ensure sound and reliable results, those laboratories should be accredited for the use of these methods according to standard EN ISO/IEC 17025.

One of the instruments to reach a high-quality standard and performance is the participation in the interlaboratory test (Proficiency Test, PT) to demonstrate that the analytical data obtained from laboratories are reliable. For this reason, it is important to organize PTs for the active ingredient content for the official laboratories.

As the national monitoring programs are in comply with the European monitoring programs, it is useful to enlarge the invitation to European Member State laboratories that work on this issue.

This activity was planned in the framework of the collaboration with the Italian Ministry of Health and the Istituto Superiore di Sanità (ISS, the National Institute of Health in Italy).

INTRODUCTION

The 5th Italian PT on PPPs (later indicated as ITPT2022) was organized between November 2021 and April 2022.

The announcement letter (Appendix A) sent to the laboratories on 4th November 2021, according to the calendar the laboratories was asked to forward the invitation. Eight Italian laboratories and to 17 European laboratories received the invitation.

All relevant Italian laboratories and European Member State laboratories participated in the ITPT2022.

In January, three different commercial products containing three active ingredients (Spinosad 44.2%; Tau Fluvalinate 21.4%; Bentazone 87.0%) were shipped to the laboratories.

Each participant received the sample bottles.

The relevant documents, such as Safety Disposable Sheets (SDS), technical instructions and the result report form were sent by e-mail. It was requested to determine the content of active ingredient using a routine analytical method applied in each participant's laboratory.

The participants were asked to report the measurement results in three significant figures in the provided result form. Also, the laboratories gave technical information on their methods, such as extractant, sample preparation, injection volume, the column used, column temperature and detector used.

The deadline of the test results submission was in April 2022.

1. PROFICIENCY TEST ON PLANT PROTECTION PRODUCTS

1.1. Test materials

The test materials of the ITPT2022 consisted of three PPPs obtained from manufacturer and available from Italian market. The product types are: Soluble Concentrate (SL), Emulsion (EW) and Water-Soluble Granule (WSG) at a declared concentration reported in Table 1.

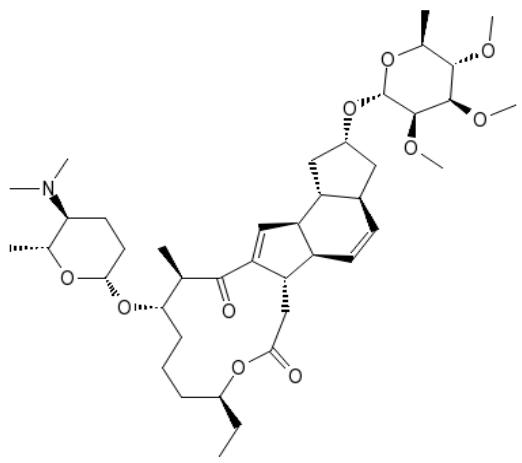
Table 1. Test materials of ITPT2022

Check Sample N.	Product description	Active ingredient	Declared level %
PPP01	Soluble Concentrate	Spinosad	44.2
PPP02	Emulsion	Tau Fluvalinate	21.4
PPP03	Water Soluble Granule	Bentazone	87

For the preparation of the subsamples to send to each laboratory, the PPPs were mixed mechanically and shared in 18 samples for a total of 54 plastic containers sealed and stored at ambient temperature before the shipment to the participants. Each laboratory received three samples, except one laboratory that analysed only Bentazone. Nothing was added to our samples.

1.2. Description of the active substances in the PPPs

1.2.1. Spinosad



Common name Spinosad

IUPAC name
(1S,2S,5R,7S,9S,10S,14R,19S)-15-[(2R,5S,6R)-5-(dimethylamino)-6-methyloxan-2-yl]oxy-19-ethyl-4,14-dimethyl-7-[(2R,3R,4R, 5S,6S)-3,4,5-trimethoxy-6-methyloxan-2-yl]oxy-20-oxatetracyclo[10.10.0.02,10.05,9]docosa-3,11-diene-13,21-dione; (1S,2R,5S,7R,9R,10S, 14R,19S)-15-[(2R,5S,6R)-5-(dimethylamino)-6-methyloxan-2-yl]oxy-19-ethyl-14-methyl-7-[(2R, 3R,4R,5S,6S)-3,4,5-trimethoxy-6-methyloxan-2-yl]oxy-20-oxatetracyclo [10.10.0.02, 10.05,9] docosa-3,11-diene-13,21-dione

Structure Formula $C_{83}H_{132}N_2O_{20}$

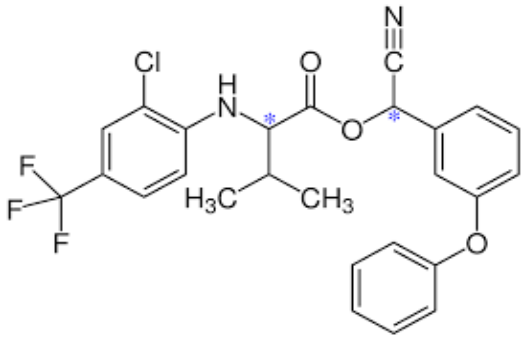
CAS number 168316-95-8

Spinosad is a mixture of Spinosyn A and Spinosyn D with molecular weight of 732.0 g/mol for Spinosyn A and 746.0 g/mol for spinosyn D.

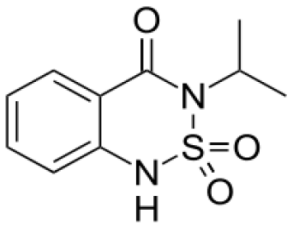
Spinosad is an insecticide based on chemical compounds found in the bacterial species Saccharopolyspora spinosa. Spinosad is a mixture

	of chemical compounds in the spinosyn family that has a generalized structure consisting of a unique tetracyclic ring system attached to an amino sugar (D-forosamine) and a neutral sugar (tri-O-methyl-L-rhamnose). Spinosad is relatively nonpolar and not easily dissolved in water. Spinosad is highly active, by both contact and ingestion, in numerous insect species. The mode of action of spinosoid insecticides is by a neural mechanism. The spinosyns and spinosoids have a novel mode of action, primarily targeting binding sites on nicotinic acetylcholine receptors (nAChRs) of the insect nervous system that are distinct from those at which other insecticides have their activity.

1.2.2. Tau Fluvalinate

	**Common name** Tau Fluvalinate **IUPAC name** (RS)-α-cyano-3-phenoxybenzyl N–(2-chloro-α,α,α-trifluoro-p-tolyl)-D-valinate **Structure formula** $C_{26}H_{22}ClF_3N_2O_3$ **CAS number** 102851-06-9 Tau Fluvalinate is a synthetic pyrethroid with molecular weight of 502.9 g/mol. Tau Fluvalinate (τ-fluvalinate) is the trivial name for (2R)-fluvalinate. The C atom in the valinate structure is in (R)-absolute configuration, while the second chiral atom is a mixture of (R)- and (S)-configurations. It is used as insecticide and acaricide with contact and stomach action. It is used to control a wide range of insects and spider mites.

1.2.3. Bentazone

	**Common name** Bentazone **IUPAC name** 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide **Structure formula** $C_{10}H_{12}N_2O_3S$ **CAS number** 25057-89-0 Bentazone is benzothiadiazinone with a molecular weight of 240.3 g/mol. It is herbicide, absorbed mainly by foliage, with very little translocation, but also absorbed by the roots, with translocation. Bentazone is used as contact herbicide. It is a selective herbicide as it only damages plants unable to metabolize the chemical. It is usually applied through contact spraying on food crops to control the spread of weeds occurring amongst food crops.

1.3. Homogeneity and stability test

Homogeneity and stability tests were performed according to the ISO 13528:2015(E) - Annex B and the International Harmonized Protocol.

For the determination of the homogeneity and stability test, the organiser used the CIPAC n. 636 method for Spinosad, CIPAC n. 366 method for Bentazone and manufacturer's method for Tau Fluvalinate.

1.3.1. Spinosad analytical method

For determination of Spinosad, weight a quantity of sample corresponding to 25 mg of Spinosad. Add 5 mL of water and 90 mL of methanol, mix allow to cool to room temperature and fill up to the mark with methanol (100 mL). Filter on 0.2 mm PTFE (polytetrafluoroethylene) filter before injection. The determination was performed using High Performance Liquid Chromatography Coupled Diode Array detector (HPLC/DAD) at 250 nm. A Zorbax SB-Aq column 150 x 4.6mm; 5 µm was used; flow rate was 1 mL/min; column temperature: 35°C; mobile phase: H_2O/acetonitrile/ammonium acetate solution (40:40:20, v/v/v).

1.3.2. Tau Fluvalinate analytical method

For determination of Tau Fluvalinate, weight a quantity of sample corresponding to 100 mg of Tau Fluvalinate into a 50-mL volumetric flask and a volume of 30 mL of acetonitrile was added. The solution was treated with ultrasounds for 40 minutes. After cooling, the volume was made up with acetonitrile. Filter on 0.2 mm PTFE filter before injection. The determination was performed using HPLC/DAD at 267 nm. A Zorbax SB-Aq column 150 x 4.6 mm; 5 µm was used; flow rate was 1 mL/min; column temperature: 25°C; mobile phase: H_2O acidify with phosphoric acid 0.001%/acetonitrile in gradient way.

1.3.3. Bentazone analytical method

For determination of Bentazone, weight a quantity of sample corresponding to 70 mg of Bentazone into a 100-mL volumetric flask and a volume of 4 mL of methanol was added. After 6 mL of buffer solution (sodium acetate buffer 0.075N) was added. Fill up to volume with mobile phase. Mix well and filter on 0.45 um PTFE filter before injection. The determination was performed using HPLC/DAD at 340 nm. A Lichrospher 100RP18 column 250 x 4.6mm; 5 µm was used; flow rate was 1 mL/min; column temperature: 25°C; mobile phase: MetOH/Sodium acetate buffer pH=6 (40:60, v/v).

1.3.4. Homogeneity

Regarding the homogeneity test, ten bottles were randomly chosen and analysed in duplicate, in two different days.

Considering that the σ_{PT} is unknown, the statistically significant differences between PT items was evaluated with the analysis of variance T-test at α=0.05, if the data series are more than two will need the Fisher Test. The T-test shows a significativity level (P) higher than 0.05 for each active substance. It is possible to say that the samples are not different one each other: they are homogeneous.

The results are shown in Table 2 for all compounds and the concentrations are in g/kg.

Table 2. Homogeneity results of the PT samples (ITPT2022)

Sample ID	Spinosad		Tau Fluvalinate		Bentazone	
	a	b	a	b	a	b
#1	446.3	453.0	225.0	225.2	885.6	882.6
#2	444.0	449.0	226.4	231.6	898.7	883.5
#3	444.0	451.4	226.1	229.0	889.0	871.7
#4	462.4	463.8	225.5	224.8	897.4	878.9
#5	466.4	473.4	227.9	228.8	892.7	879.3
#6	445.5	450.6	225.2	230.9	892.3	886.5
#7	460.0	466.9	226.1	231.3	890.1	900.3
#8	447.8	449.6	225.6	226.5	910.9	903.5
#9	438.7	437.3	225.8	226.4	891.1	886.4
#10	448.6	456.5	224.4	224.5	902.3	899.4
Mean	450.3	455.1	225.8	227.9	895.0	887.2
SD	9.2	10.4	1.0	2.8	7.5	10.5
t**						
P***						
Homogeneity	YES		YES		YES	

a, b: replicates of the same sample
t**: T of Student Test
P***: significativity level;
SD: Standard Deviation

1.3.5. Stability

The stability test was performed using two bottles, randomly chosen, which were analysed in duplicate in two occasions and each occasion twice:

- *Day 1*: before the shipment of the samples on January 2022;
- *Day 2*: at the deadline for reporting results on April 2022.

Stability test was judged acceptable as the percentage difference of concentration, for each active substance was found less than 10%.

Table 3 shows the stability data of the ITPT2022.

Table 3. Summary of stability data in g/kg (ITPT2022)

Active ingredient	January	April	Concentration
Spinosad	448	461	443
Tau Fluvalinate	231	235	240
Bentazone	892	905	870

Tables 4, 5, and 6 show the individual results for each substance. The deviation calculated with reference to the 1st analysis and to the declared label shows a deviation less than 10% for all substances. The products are stable.

Table 4. SPINOSAD: results of stability test (ITPT2022)

Parameter	January				April			
	Replicate 1		Replicate 2		Replicate 1		Replicate 2	
	inj 1	inj 2	inj 1	inj 2	inj 1	inj 2	inj 1	inj 2
Sample 1	447.1	443.3	450.9	441.8	455.2	454.8	454.5	454.5
Sample 2	451.8	447.1	453.3	445.0	465.4	466.0	467.8	467.8
Mean	447.3		447.8		460.4		461.2	
SD	3.5		5.3		6.2		7.7	
Mean of 2 days	447.5				460.8			
Standard Deviation of 2 days	4.1				6.5			
Deviation (ref 1st Analysis)/ [(M2-M1)/M1]*100	2.9							
Deviation (ref to declared label g/kg)/ [(SM-443)/443]*100	2.5							
Stability Mean	454.2		Declared Label g/kg		443			
Stability Standard Deviation	9.4		CV %		2.1			

Table 5. TAU FLUVALINATE: results of stability test (ITPT2022)

Parameter	January				April			
	Replicate 1		Replicate 2		Replicate 1		Replicate 2	
	inj 1	inj 2	inj 1	inj 2	inj 1	inj 2	inj 1	inj 2
Sample 1	235.8	234.2	229.3	229.1	233.9	233.4	232.4	249.4
Sample 2	229.7	229.3	229.3	229.3	233.8	235.3	231.7	231.9
Mean	232.3		229.3		234.1		236.4	
SD	3.2		0.1		0.8		8.7	
Mean of 2 days	230.8				235.2			
Standard Deviation of 2 days	2.1				1.6			
Deviation (ref 1st Analysis)/ [(M2-M1)/M1]*100	1.9%							
Deviation (ref to declared label g/kg)/ [(SM-240)/240]*100	2.9%							
Stability Mean	233.0		Declared Label g/kg		240			
Stability Standard Deviation	3.1		CV %		1.3			

Table 6. BENTAZONE: results of stability test (ITPT2022)

Parameter	January				April			
	Replicate 1		Replicate 2		Replicate 1		Replicate 2	
	inj 1	inj 2	inj 1	inj 2	inj 1	inj 2	inj 1	inj 2
Sample 1	895.5	898.9	890.5	894.6	900.1	900.4	898.1	900.1
Sample 2	894.6	891.1	886.8	886.0	909.1	912.1	909.5	909.9
Mean	895.0		889.5		905.4		904.4	
SD	3.2		3.9		6.10		6.2	
Mean of 2 days	892.3				904.9			
Standard Deviation of 2 days	4.5				5.7			
Deviation (ref 1st Analysis)/ [(M2-M1)/M1]*100	1.4							
Deviation (ref to declared label g/kg)/ [(SM-870)/870]*100	3.3							
Stability Mean (SM)	898.6			Declared Label g/kg	870			
Stability Standard Deviation	8.2			CV %	0.9			

1.4. Distribution of the samples and instructions for the participants

Three plastic transparent containers with red cup were filled. Each sample was shipped to the participating laboratories at ambient temperature. An information message was sent out by e-mail during shipment so that laboratories make their own arrangements for the reception of the package, and a protocol was sent by e-mail.

The participants (Appendix B) were asked:

– to inform on the safe recipient of the samples in their laboratories;
– to report results in the appropriate form and send them to the organizer by e-mail along with the details of methodology used.

The samples were sent to the participants on 15th January 2022.

The deadline for results was 30th of April 2022.

The final report was dispatched to all participants at the end of July 2022.

1.5. Statistical evaluation of results

This PT has been evaluated using the modified z-score parameter to rate the laboratory performance for each active substance according to AAPCO protocol.

The outliers were calculated using the modified z-score.

A Horwitz ratio (HorRat) has been calculated. The HorRat is a normalized performance parameter indicating the acceptability of analytical methods respect to among-laboratory precision (reproducibility). It illustrates the deviation or agreement of an observed interlaboratory

reproducibility with typical values. In this PT, the HorRat for the laboratories was calculated for each substance.

1.5.1. Robust mean

The purpose of using a robust estimator for the mean was to cope with the possibility of outlying data points without having to remove them from the sample.

The robust mean estimator used was the median.

1.5.2. Robust estimate of standard deviation

The robust estimate of the standard deviation used was the MAD_E value.

To obtain the MAD_E, calculate Median Absolute Deviation (MAD) from the sample median:

$$MAD = median\ (|X_i - median\ (Xi)|_{i=1,2\ldots n})$$

Calculate MAD_E:

$$MAD_E = K \times MAD$$

For normally distributed data, K= 1.483:

$$MAD_E = 1.483 \times MAD$$

1.5.3. Calculation of modified z-scores

Modified z-scores (Zi) for each laboratory were calculated as:

$$Zi = 0.6745 \times {}^{(Xi - median)} / MAD$$

Z values falling outside the range of $-3.5 \leq Zi \leq 3.5$ were marked as outliers.

1.5.3. Calculation of Horwitz ratio

A HorRat can be calculated using RSD (Relative Standard Deviation), which is defined as follow:

$$HorRat = RSDr/PRSDr$$

where RSDr is the RSD among laboratories and PRSDr is the predicted standard deviation from Horwitz equation:

$$PRSDr(\%) = 2C^{-0.15}$$

where C is the concentration found expressed as a mass fraction.

The empirical acceptable HorRat value is ranged between 0.5-2.0.

1.5.4. Presentation of data

Data is presented graphically in two ways:

– A scatter plot showing each participating laboratory's two-day mean value for each analyte along with the associated standard deviation. These plots also show the upper and lower Horwitz (Thompson) limits for the sample, as well as median ±2 MAD_E.

– A plot of modified z-scores.

2. ANALYSIS OF THE SUBSTANCES

Description and statistical evaluation of the results are presented for each compound separately.

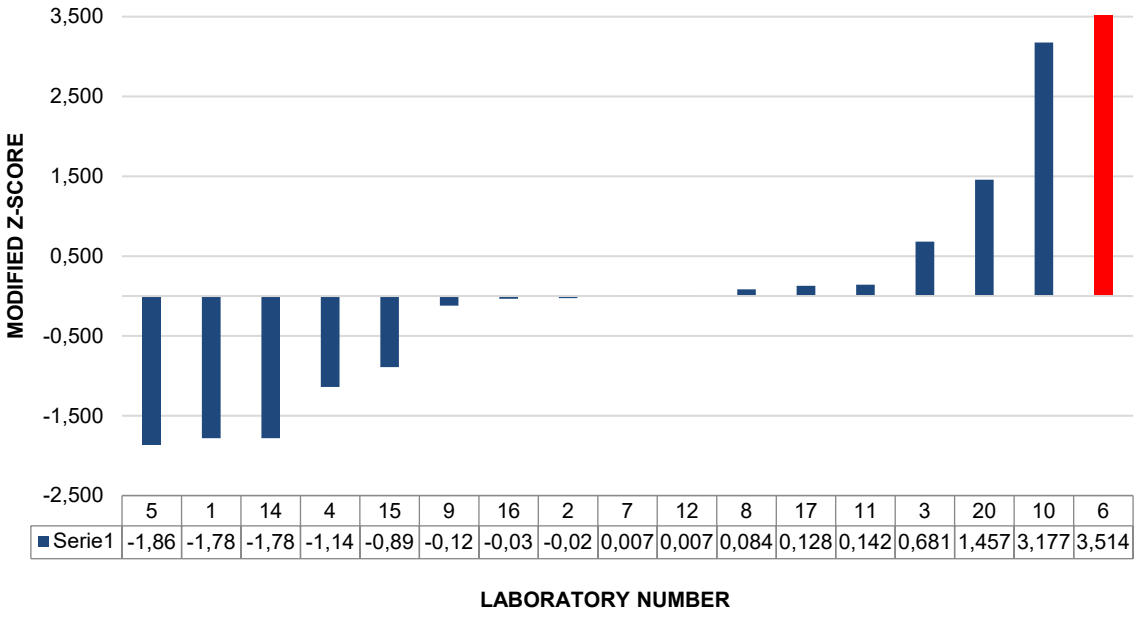
2.1. Spinosad

For the active substance Spinosad, 22 boxes were sent to all laboratories, in particular 5 to Italian Laboratories and 17 to European Laboratories outside Italy. We received 17 participation results. Sixteen laboratories used for the analysis an HPLC instrument with a UV Detector and 1 with a Mass Spectrometry (MS) Detector.

To carry out this analysis 7 laboratories applied an in-house method, 7 the CIPAC method and 2 used the manufacturer's method, as is showed in Table 7. At the same time, all the methods gave appreciable data, as Figure 1 shows.

Table 7. SPINOSAD: methods applied for analysis (ITPT2022)

Laboratories	In-house	CIPAC	Manufacturer's
Number	7	7	2



	5	1	14	4	15	9	16	2	7	12	8	17	11	3	20	10	6
Serie1	-1,86	-1,78	-1,78	-1,14	-0,89	-0,12	-0,03	-0,02	0,007	0,007	0,084	0,128	0,142	0,681	1,457	3,177	3,514

Figure 1. SPINOSAD: modified z-scores (ITPT2022)

For Spinosad, a statistical evaluation based on a robust estimator instead of the mean was applied on the collected data. The purpose of this choice was to cope with the possibility of

outlying data points without having to remove them, so the median and the standard deviation were used. Figure 1 shows the lab's values of modified z-score. The results obtained are valuable data, in fact most of them are inside the z-score range of $-3.5 \leq Z \leq +3.5$ except one with a z-score value of 3,514.

The HorRat value calculated was 1.9 that indicates good acceptability of the chemical methods used with respect to precision.

2.2. Tau Fluvalinate

For the substance Tau Fluvalinate, 22 boxes were sent to laboratories, in particular 5 to Italian Laboratories and 17 to European Laboratories outside Italy. We received 17 participation results. The analysis was performed using LC instrument for 16 laboratories with UV Detector and one decided to use the Gas Chromatography (GC) with Flame Ionisation Detector (FID). Eleven laboratories chose to use an in-house method, others six a manufacturer's method, as is showed in Table 8. For this substance no CIPAC method exists. At the same time, all the methods gave appreciable data.

Table 8. TAU FLUVALINATE: methods applied for analysis (ITPT2022)

Laboratories	In-house	CIPAC	Manufacturer's
Number	11	0	6

As for the Spinosad mentioned before, a statistical evaluation based on a robust estimator (median) instead of the mean was applied on the collected data. The purpose of this choice was to cope with the possibility of outlying data points without having to remove them, so the median and the standard deviation were used. Figure 2 shows the lab's values of modified z-score.

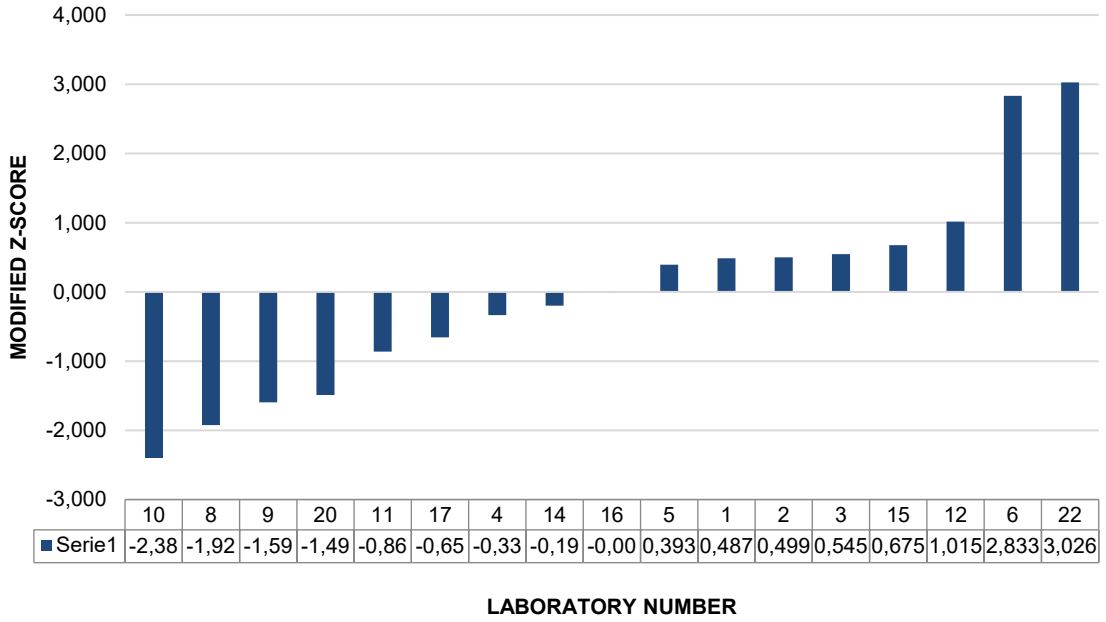


Figure 2. TAU FLUVALINATE: modified z-scores (ITPT2022)

The results obtained are valuable data, in fact all of them are inside the z-score range of -3.5 ≤ Z ≤ +3.5.

One laboratory obtained the excellent value of modified z-score of 0.

The HorRat value calculated was 1.9 that indicates good acceptability of the chemical methods used with respect to precision.

2.3. Bentazone

Regarding the active substance Bentazone, 22 boxes were sent worldwide, in particular 5 to Italian Laboratories and 17 to European Laboratories outside Italy. We received 18 participation results; four laboratory missing results. All the laboratories used for the analysis an LC instrument with a UV Detector. It is interesting to note that almost all of the laboratories chose to use an in-house method, 5 laboratories choose to use CIPAC methods, one applied a manufacturer's method and just and just applied a published method, as is showed in Table 9. At the same time, all the methods gave appreciable data.

Table 9. BENTAZONE: methods applied for analysis (ITPT2022)

Laboratories	In-house	CIPAC	Manufacturer's
Number	12	5	1

On the collected data, a statistical evaluation was applied based on a robust estimator (median) instead the mean. The purpose of this choice was to cope the possibility of outlying data points without having to remove them, so it was used the median and the standard deviation.

Figure 3 shows the lab's values of modified z-score. The results obtained are laudable data, in fact all of them are inside the modified z-score range of -3.5 ≤ Z ≤ +3.5.

The HorRat value calculated was 1.0 that indicates good acceptability of the chemical methods used with respect to precision.

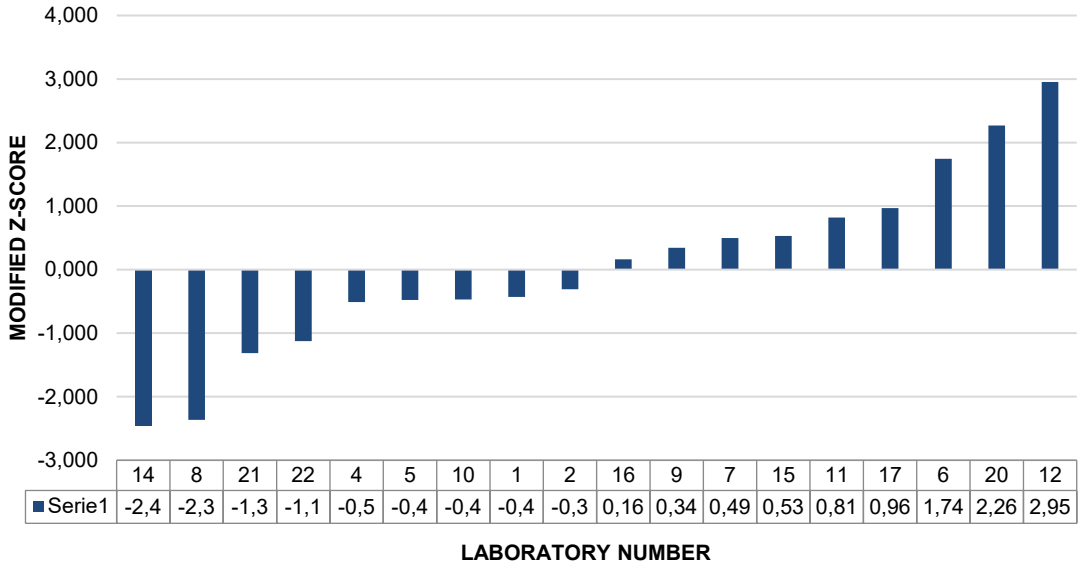


Figure 3. BENTAZONE: modified z-scores (ITPT2022)

3. RESULTS

The results of the 5th ITPT2022 can be regarded as satisfactory.

The high proportion of laboratories invited that became participants demonstrated a good interest of the laboratories in this exercise. The participation of the Italian and European laboratories was good. For Italy, four laboratories participated so distributed: two from the north, one from the centre and one from the south of the Country. The European laboratories were thirteen, excluding Italy, distributed throughout Europe.

For the analysis of Spinosad, 47% of the laboratories used the CIPAC method and 41% used in-house methods.

As concerns Tau Fluvalinate, 65% of the laboratories used in-house methods, 35% manufacturer's method and none CIPAC method because not available.

Finally, for Bentazone, 66.7% of the laboratories used in-house methods 27.8% CIPAC method and 5.5% manufacturer's method.

Most of the laboratories used LC technique and only one used GS technique for Tau Fluvalinate as shown in Table 10.

Only for Spinosad there was a 5.8% of failing results obtained with the modified z-score, as Table 10 shows.

Table 10. Summary of participation per active ingredient (ITPT2022)

ID Sample	Product description	Active ingredient	Participants (n.)	Labs using GC (n.)	Labs using LC (n.)	Failing results[1](%)
PPP01	Soluble Concentrate	Spinosad	17	0	17	5.8
PPP02	Emulsion	Tau Fluvalinate	17	1	16	0
PPP03	Water soluble granule	Bentazone	18	0	18	0

[1] Where failing indicates a mean assay result outside the modified z-score defined acceptable limits.

For each substance, the HorRat value calculated indicates the good acceptability of the chemical methods used with respect to the precision.

Tables 11 and 12 summarize the results per active ingredient including and excluding the outliers.

Table 11. Summary of lab results per active ingredient, including outliers (ITPT2022)

ID Sample	Analyte (Label claim)	Minimum result	Maximum result	Grand Average	Grand %CV
PPP01	Spinosad (44.2%)	43.23	51.20	46.1	4.88
PPP02	Tau Fluvalinate (21.4%)	20.65	25.27	22.69	5.55
PPP03	Bentazone (87%)	85.43	92.15	88.5	2.08

Table 12. Summary of lab results per active ingredient, excluding outliers

ID Sample	Analyte (Label claim)	N. of outliers[1]	Average excluding outliers	%CV excluding outliers
PPP01	Spinosad (44.2%)	1	45.9	0.02
PPP02	Tau Fluvalinate (21.4%)	0	no	no
PPP03	Bentazone (87%)	0	no	no

[1] An outlier is flagged when the modified z-score falls outside the range of -3.5 ≤ Zi ≤ 3.5; see Appendix for calculations.

The performance of the laboratories in terms of modified z-score was satisfactory for almost all participants for all substances. For all active substance, there are not outlier values.

Based on the results, we can conclude that the PT was successful and that a satisfactory number of laboratories participated despite the COVID-19 pandemic.

Tables 13, 14 and 15 show details of the z-score values for each laboratory and the analytical technique used for each substance.

Table 16, 17 and 18 report the information on analytical methods used for each substance and each laboratory.

Table 13. SPINOSAD Sample PPP01: summary results (ITPT2022)

Lab #	Method	Two Day Average[1]	RPD[1]	Modified Z Score[2]	Outlier[2]
1	HPLC/UVD	43.4	1.15	-1.781	NO
2	HPLC/UV	46.0	1.52	-0.027	NO
3	HPLC/UV	47.0	0.00	0.681	NO
4	HPLC/DAD	44.3	1.35	-1.140	NO
5	HPLC/DAD	43.2	0.46	-1.862	NO
6	HPLC/DAD	51.2	2.34	3.514	YES
7	HPLC/DAD	46.0	1.30	0.007	NO
8	HPLC/MS	46.1	-3.79	0.084	NO
9	HPLC/DAD	45.8	2.05	-0.121	NO
10	HPLC/DAD	50.7	-0.79	3.177	NO
11	HPLC/DAD	46.2	3.46	0.142	NO
12	HPLC/DAD	46.0	-2.17	0.007	NO
14	HPLC/DAD	43.4	-0.69	-1.781	NO
15	HPLC/DAD	44.7	0.40	-0.890	NO
16	HPLC/DAD	45.9	-0.87	-0.034	NO
17	HPLC/UV	46.2	0.74	0.128	NO
20	HPLC/DAD	48.2	-0.04	1.457	NO

Grand Average[3]	46.1
Total SD	2.3
Total Median[4]	46.0
MAD	0.01
MAD_E	0.01

[1] Average yield and Relative Percent Difference between the two-day determinations per laboratory.
[2] An outlier is flagged when the modified z-score falls outside the range of -3.5 ≤ Z ≤ 3.5; *see* Glossary.
[3] Grand average, standard deviation and median.
[4] Median Absolute Deviation Robust estimation of standard deviation; *see* Appendix for calculations.

Table 14. TAU FLUVALINATE Sample PPP02: summary results (ITPT2022)

Lab #	Method	Two Day Average[1]	RPD[1]	Modified Z Score[2]	Outlier[2]
1	HPLC/UV	23.1	0.00	0.487	NO
2	GC/FID	23.1	-2.16	0.499	NO
3	HPLC/UV	23.2	-2.16	0.545	NO
4	HPLC/DAD	22.4	-0.89	-0.334	NO
5	HPLC/DAD	23.0	0.00	0.393	NO
6	HPLC/DAD	25.1	-7.17	2.833	NO
8	HPLC/UV	21.1	-1.38	-1.924	NO
9	HPLC/DAD	21.3	0.70	-1.595	NO
10	HPLC/DAD	20.7	1.45	-2.387	NO
11	HPLC/DAD	21.9	-0.46	-0.862	NO
12	HPLC/DAD	23.6	-1.27	1.015	NO
14	HPLC/DAD	22.5	-1.11	-0.199	NO
15	HPLC/DAD	23.2	-3.27	0.679	NO
16	HPLC/VWD	22.7	0.44	-0.006	NO
17	HPLC/PDA	22.1	0.05	-0.657	NO
20	UPLC/DAD	21.4	0.70	-1.490	NO
22	HPLC/UV	25.3	-0.20	3.026	NO

Grand Average[3]	22.69
Total SD	1.28
Total Median[4]	22.68
MAD	0.006
MAD_E	0.009

[1] Average yield and Relative Percent Difference between the two-day determinations per laboratory.
[2] An outlier is flagged when the modified z-score falls outside the range of -3.5 ≤ Z ≤ 3.5; *see* Glossary.
[3] Grand average, standard deviation and median.
[4] Median Absolute Deviation Robust estimation of standard deviation; *see* Appendix for calculations.

Table 15. BENTAZONE Sample PPP03: summary results (ITPT2022)

Lab #	Method	Two Day Average[1]	RPD[1]	Modified Z Score[2]	Outlier[2]
1	HPLC/UV	87.9	0.34	-0.429	NO
2	HPLC/UV	88.1	-0.68	-0.308	NO
4	HPLC/DAD	87.9	1.71	-0.509	NO
5	HPLC/DAD	87.9	0.30	-0.477	NO
6	HPLC/DAD	90.7	-3.20	1.746	NO
7	HPLC/DAD	89.1	-0.45	0.497	NO
8	HPLC/UV	85.6	0.36	-2.366	NO
9	HPLC/DAD	88.9	0.02	0.344	NO
10	HPLC/DAD	87.9	0.91	-0.469	NO
11	HPLC/DAD	89.5	2.01	0.819	NO
12	HPLC/DAD	92.2	3.80	2.954	NO
14	HPLC/DAD	85.4	-0.44	-2.458	NO
15	HPLC/DAD	89.1	0.00	0.530	NO
16	HPLC/VWD	88.7	-0.80	0.163	NO
17	HPLC/PAD	89.7	0.26	0.968	NO
20	HPLC/DAD	91.3	0.00	2.269	NO
21	HPLC/DAD	86.9	-0.35	-1.315	NO
22	HPLC/DAD	87.1	-2.26	-1.126	NO

Grand Average[3]	88.54
Total SD	1.90
Total Median[4]	88.39
MAD	0.008
MAD_E	0.012

[1] Average yield and Relative Percent Difference between the two-day determinations per laboratory.
[2] An outlier is flagged when the modified z-score falls outside the range of -3.5 ≤ Z ≤ 3.5; *see* Glossary.
[3] Grand average, standard deviation and median.
[4] Median Absolute Deviation Robust estimation of standard deviation; *see* Appendix for calculations.

Table 16. SPINOSAD: representative method for the determination (ITPT2022)

ID Lab	Reference method	Internal standard	Extraction solvent	Sample preparation	Injection volume µL	Column T°	Detector	Stationary phase (SP) Mobile phase (MP)
1	In house method	No	Acetonitrile	Weight a sample portion diluted in ACN, sonicate for 15 min, filter through a 0.45 µm filter	5	Ambient	UVD 254 nm	**SP**: Zorbax XDB 250 X 3mm; 5 µm; 0.5 mL/min **MP**: Acetonitrile/H_2O acidified H_3PO_4
2	In-house method	No	Acetonitrile/water (9:1)	Sonicate for 8 min, filter through 0.22 µm PTFE syringe filter	0.5	25°C	UV 240 nm	**SP**: Phenomenex Kinetex C18; 100 X 2.1 mm; 2.6 µm; 0.4 mL/min **MP**: Acetonitrile/H_2O acidified 0.1% H_3PO_4
3	In house method	No	Acetonitrile	Weight a sample portion diluted in ACN, sonicate for 15 min.	2	30°C	UV 246 nm	**SP**: Xterra RP18 150 X 2.1 mm; 3.5 µm; 0.3 mL/min **MP**: Acetonitrile/H_2O 0.1% acidified H_3PO_4
4	CIPAC 636	No	MilliQ water and methanol	Ultrasonicate for 30 min in MilliQ-water. Dilute with methanol and filter through 0.45 mm PTFE-filter	20	25°C	DAD 250 nm	**SP**: Luna C18(2) 250 X 4.6mm; 5 µm; 1.5 mL/min **MP**: methanol/acetonitrile ammonium acetate solution 20 g/L pH5.3 with acetic acid
5	CIPAC	No	Water HPLC Methanol HPLC	Dilute a portion of sample in 100 mL volumetric flasks with 5 mL water HPLC and 95 mL methanol HPLC	20	35°C	DAD 250 nm	**SP**: Partisil 5 ODS 3 100 X 4.6 mm; 5 µm; 1.0 mL/min **MP**: methanol/acetonitrile/ammonium acetate solution (40+40+20, v/v) isocratic
6	CIPAC	No	Methanol	Sonicate for 15 mins. Filtrate through 0.2 µm PP disk	20	35°C	DAD 245 nm	**SP**: Kromasil C8 150 X 4.6 mm; 5 µm; 0.7 mL/min **MP**: methanol/acetonitrile (50/50, v/v) (80%) + ammonium acetate solution 20 g/L pH5.3 (20%)

to be followed

continues

ID Lab	Reference method	Internal standard	Extraction solvent	Sample preparation	Injection volume µL	Column T°	Detector	Stationary phase (SP) Mobile phase (MP)
7	In-house method (BVL_A-02 Anhang W-1008-01)	No	Methanol	Weight a portion of sample. Dissolve in 50 mL methanol, Sonicate for 15 min Dilute with methanol (1:4 ratio)	10	35°C	DAD 250 nm	**SP**: Lichrospher 100 RP18 250 X 4 mm; 5 µm; 2.0 mL/min **MP**: methanol/acetonitrile/ammonium acetate buffer pH 5.3 (44/44/12)
8	In-house method	No	Methanol/water (90:10)	Dissolve sample in 5 mL of water. Fill to mark with methanol. Dilute with methanol and filtrate though 0.45 µm filter.	10	25°C	MS	**SP**: Phenomenex Gemini C18 150 X 2 mm; 3 µm; 0.2 mL/min **MP**: methanol/water acid. 0.3% formic acid
9	In-house method	No	Water + Acetonitrile	Sonicate for 30 min. Filtrate through 0.45 µm PTFE disk	10	40°C	DAD 245 nm	**SP**: Zorbax ODS 150 X 4.6mm; 5 µm; 0.8 mL/min **MP**: Acetonitrile/H_3PO_4 0.5% (60:40, v/v)
10	Manufacturer's method	No	Methanol	Weight a portion of sample add 2 mL H_2O / 25 mL CH_3OH Dilute 10 mL with 25 mL of CH_3OH	20	NA	DAD 254 nm	**SP**: Zorbax Eclipse Plus C18 150 X 4.6mm; 5 µm; 1 mL/min **MP**: Acetonitrile/CH_3OH/H_2O buff (500 mL H_2O + 10 g ammonium acetate) (45/45/10, % v/v/v)
11	CIPAC 636SC/(M)/3	No	Methanol	Dissolve the sample in 100 mL of methanol. Sonicate for 10 minutes. Transfer 10 mL of this solution in a 100 mL volumetric flask. Filter through a 0.45 µm PTFE filter	20	35°C	PDA 250 nm	**SP**: YMC Pack ODS-AQ 150 X 4.6 mm; 5µm; 1.5 mL/min **MP**: methanol/acetonitrile/ammonium acetate solution (40+40+20, v/v)
12	In-house method	No	Acetonitrile	Weight a sample portion, add acetonitrile. Sonicate 15 min. Filtrate on 0.45 µm nylon filter	10	35°C	DAD 254 nm	**SP**: Zorbax Eclipse C8 150 X 4.6 mm; 5 µm; 1.5 mL/min **MP**: Acetonitrile/H_3PO_4 0.1%

to be followed

continues

ID Lab	Reference method	Internal standard	Extraction solvent	Sample preparation	Injection volume µL	Column C°	Detector	Stationary phase (SP) Mobile phase (MP)
14	CIPAC MT 636	No	Water/methanol	Suspend in aprox 10 mL of water and fill to 100 mL with methanol. Sonicate 10 min. Dilute 1/10 with methanol and filter through 0.45 µm PTFE disk	20	35°C	DAD 250 nm	**SP**: Luna C18(2) 150 X 4.6 mm; 5 µm; 1.2 mL/min **MP**: methanol/acetonitrile/ 2% ammonium acetate solution pH 5.3 (40:40:20)
15	CIPAC MT 636	No	Methanol	Dilute with methanol. Sonicate for 3 minutes. Filter through 0,2 µm PP disk before injection	20	35°C	DAD 250 nm	**SP**: Zorbax SB-Aq 150 X 4.6 mm; 5 µm; 1.0 mL/min **MP**: Water/acetonitrile/ammonium acetate solution (40:40:20, v/v/v)
16	In-house method	No	Water/Acetonitrile (1:24)	Sonicate 10 min. Filtrate through Nylon 0.45 µm disk	5	30°C	VWD 230 nm	**SP**: Zorbax Eclipse, XDB C18; 250 X 4.6 mm; 5 µm; 1.1 mL/min **MP**: Acetonitrile/H_2O acidified 0.1% H_3PO_4 (gradient)
17	Manufacturer's method	No	Methanol	Weight a sample portion. Add solvent and sonicate, make up to volume, dilute 1:10	20	35°C	DAD 250 nm	**SP**: Inertisil ODS3 C18 250 X 4.6 mm; 5 µm; 1.5 mL/min **MP**: methanol/acetonitrile/ ammonium acetate solution (44/44/12)
20	CIPAC	No	Methanol	Weight a sample portion diluted in 100 mL methanol, sonicate for 30 min; filtrate on 0.2 µm filter in vial	5	40°C	DAD 250 nm	**SP**: Luna C18 150 X 4.6 mm; 5 µm; 1.5 mL/min **MP**: methanol/acetonitrile (40/40, %) 15 mM ammonium acetate solution pH 5.3 (20%)

Table 17. TAU FLUVALINATE: representative method for the determination (ITPT2022)

ID Lab	Reference method	Internal standard	Extraction solvent	Sample preparation	Injection volume µL	Column T°	Detector	Stationary phase (SP) Mobile phase (MP)
1	In-house method	No	Acetonitrile	Sonicate for 15 min. Filtrate through 0.45 µm filter.	5	25	UV 254 nm	**SP**: Zorbax XDB-C18 250 x 3 mm; 5 µm; 0.5 mL/min **MP**: water phosphoric acid/acetonitrile
2	In-house method	Dicyclohexylphtalate, 0.08 mg/mL	Acetone	Sonicate for 8 min. filtrate through 0.45 µm PTFE filter.	1	280°C hold 8 min	FID	**SP**: HP-1 5 m x 0.53 mm; 2.65 µm; Isothermal Injector temperature: 250°C; Injection ratio: Split, 5:1 **MP**: Nitrogen
3	In-house method	No	Acetonitrile	Sonicate for 15 min.	2	30	UV 260 nm	**SP**: Xterra RP18 150 x 2.1 mm; 3.5 µm; 0.3 mL/min **MP**: Water acid phosphoric acid 0.1%/Acetonitrile
4	Manufacturer's method	NA	Water/Acetonitrile (10:90)	Sonicate for 5 min in MilliQ-water and for 5 min with acetonitrile. Diluted and filtrate through 0.45 µm PTFE-filter.	10	20	DAD 254 nm	**SP**: Primesphere C8 150 x 4.6 mm; 5 µm; 1.5 mL/min **MP**: Water/Acetonitrile/Methanol
5	In-house method	NA	Water/Acetonitrile (5:95)	Dilute a quantity of sample in 100 mL volumetric flasks with 5 mL water HPLC grade and 95 mL acetonitrile. Dilute 5mL to 10 mL volumetric flask with Acetonitrile	5	35	DAD 254 nm	**SP**: Zorbax SB-C8 150 x 4.6 mm; 3.5 µm; 1.2 mL/min **MP**: Water /Acetonitrile/Methanol (20:40:40, v/v/v)
6	In-house method	NA	Acetone	Sonicate for 15 min. Filtrate through 0.2 µm PP disk	10	35	DAD 210 nm	**SP**: Kinetex Phenomenex 150 x 4.6 mm; 2.6 µm; 1.3 mL/min **MP**: Water /Acetonitrile (20:80, v/v)

to be followed

continues

ID Lab	Reference method	Internal standard	Extraction solvent	Sample preparation	Injection volume µL	Column T°	Detector	Stationary phase (SP) Mobile phase (MP)
8	In-house method	No	Methanol/Water 90:10	Dissolve sample in 5mL of water. Fill to mark with methanol. Diluted with methanol and filtrate through 0.45 filter.	10	25	UV 280 nm	**SP**: Phenomenex Gemini C18 150 x 2 mm; 3 µm; 0.2 mL/min **MP**: Water acid Formic acid 0.3% /CH_3OH
9	In-house method	No	Acetonitrile	Sonicate for 30 min. Filtrate through 0.45 µm PTFE disk	5	40	DAD 255 nm	**SP**: Phenomenex Luna 150 x 4.6 mm; 3 µm; 1.2 mL/min **MP**: Water acid Phosphoric acid 0.1% /Acetonitrile (10:90, v/v)
10	Manufacturer's method	No	Acetonitrile	Weight sample and dilute in 25 mL acetonitrile	5	NA	DAD 205 nm	**SP**: Zorbax Eclipse Plus C18 250 x 4.6 mm; 5 µm; 0.5 mL/min **MP**: Water acidify phosphoric acid /Acetonitrile (10/90, v/v)
11	Manufacturer's method	No	Acetonitrile	Dissolve the sample in 100 mL of acetonitrile. Sonicate for 10 minutes. Filtrate through a 0.45 µm PTFE filter.	5	40	DAD 254 nm	**SP**: Agilent Zorbax SB-C18 150 x 4.6 mm; 5 µm; 1.0 mL/min **MP**: Water/MeOH/Acetonitrile (20+40+40 v/v/v)
12	In-house method	No	Acetonitrile	Sonicate for 15 min. Filtrate on 0.45 nylon filter.	10	35	DAD 254 nm	**SP**: Zorbax Eclipse C8 150 x 4.6 mm; 5 µm; 1.5 mL/min **MP**: water phosphoric acid 0.1%/Acetonitrile
14	In-house method	No	Acetonitrile	Weight sample and add 3mL of water. Fill to 100mL with acetonitrile. Sonicate for 5 min. Shake and filtrate through 0.45 µm PTFE disk	5	40	DAD 260 nm	**SP**: Kinetex Phenomenex C18 100A 100 x 4.6 mm; 2.6 µm; 1.0 mL/min **MP**: Water /Acetonitrile (25:75, v/v)

to be followed

continues

ID Lab	Reference method	Internal standard	Extraction solvent	Sample preparation	Injection volume µL	Column T°	Detector	Stationary phase (SP) Mobile phase (MP)
15	Manufacturer's method	No	Acetonitrile	Dilute the sample with water and acetonitrile (6:94 v/v); sonicate for 40 minutes; filtrate through 0.2 µm PP disk before injection.	10	25	DAD 267 nm	**SP**: Zorbax SB-Aq 150 x 4.6 mm; 5 µm; 1.0 mL/min **MP**: water phosphoric acid 0.001%/Acetonitrile
16	In-house method	No	Acetonitrile:Water (24:1, v/v)	Sonicate for 10 min. Filtrate through Nylon 0.45 µm disk	5	30	VWD 230 nm	**SP**: Zorbax Eclipse XDB-C18 250 x 4.6 mm; 5 µm; 1.1 mL/min **MP**: water phosphoric acid 0.1%/Acetonitrile
17	In-house method	No	Acetonitrile	Weight sample and dilute to volume with acetonitrile	5	25	PDA 260 nm	**SP**: Kinetix C18 100A 100 x 4.6 mm; 2.6 µm; 1 mL/min **MP**: Water (0.1% formic acid)/Acetonitrile (35:65 v/v)
20	Manufacturer's method	No	Acetonitrile	Weight Sample and dilute with 50 mL acetonitrile. Sonicate for 30 min. Filtrate with 0.2 µm filter in vial	1	40	DAD 254 nm	**SP**: Byphenyl 100 x 2.1 mm; 2.6 µm; 0.3 mL/min **MP**: Water/CH_3OH/Acetonitrile (20+40+40 v/v/v)
22	Manufacturer's method	No	400 mL Methanol + 400 mL Acetonitrile + 0.5 mL acetic acid	Dilute sample in 2-3 mL water, add extraction solvent, sonicate for 15 min	2	NA	UV 254 nm	**SP**: Zorbax SB-C8 250 x 4.6 mm; 5 µm; 2 mL/min **MP**: water acetic acid 0.5 mL/CH_3OH/Acetonitrile (20+40+40 v/v/v)

Table 18. BENTAZONE: representative method for the determination (ITPT2022)

ID Lab	Reference method	Internal standard	Extraction solvent	Sample preparation	Injection volume µL	Column T°	Detector	Stationary phase (SP) Mobile phase (MP)
1	In-house method	No	Acetonitrile	Sonicate for 15 min. Filtrate through a 0.45 µm filter.	5	25	UVD 230 nm	**SP**: Zorbax XDB C18, 250 x 3 mm; 5 µm 0.5 mL/min **MP**: Acetonitrile/H_2O acid Phosphoric acid
2	In-house method	No	Acetonitrile-Water (9:1)	Sonicate for 8 min. Filtrate through 0.22 µm PTFE syringe filter	0.5	25	UV 240 nm	**SP**: Kinetex C18, 100 x 2,1 mm; 2,6 µm 0.4 mL/min **MP**: Acetonitrile/H_2O phosphoric acid 0.1%
4	CIPAC	No	10% Milli-Q-water/ 90% acetonitrile	Sonicate 15 min. Dilute and filtrate through with 0.45 µm PTFE filter	10	30	DAD 340 nm	**SP**: Kinetex C18, 150 x 4,6 mm; 5 µm 1.0 mL/min **MP**: Acetonitrile/H_2O acid formic acid 0.1%
5	CIPAC	No	2mL Methanol (HPLC grade) + 3 mL sodium acetate buffer +45 mL mobile phase	Dilute sample in 50 mL volumetric flasks with 2mL Methanol (HPLC grade) + 3 mL sodium acetate buffer +45 mL mobile phase	10	30	DAD 340 nm	**SP**: Phenomenex Luna C18, 250 x 4,6 mm; 5µm; 0.7 mL/min **MP**: Methanol/Sodium acetate buffer (40:60)
6	In-house method	No	Acidified water (0,5% H3PO4) / Tetrahydrofuran (50/50 (v/v))	Sonicate for 15 min. Filtrate through 0.2 µm PP disk.	10	35	DAD 225 nm, 302 nm	**SP**: Kinetex Phenomenex 150 x 4,6 mm; 5 µm. 0.3 mL/min **MP**: Acetonitrile/H_2O phosphoric acid 0.5%
7	In-house method	No	Methanol/ sodium acetate buffer pH6 (40/60)	Dilute in 8 mL methanol, 12 mL sodium acetate buffer pH6, add to 200 mL with mixture of methanol and sodium acetate buffer pH6 (40/60). Sonicate for 15 min.Dilute 1:2 with mixture of methanol and sodium acetate buffer pH6 (40/60)	2	30	DAD 340 nm	**SP**: LiChrospher 100 RP18, 250 x 4 mm; 5µm; 1.0 mL/min **MP**: Methanol/Sodium acetate buffer pH=6 (40:60)

to be followed

continues

ID Lab	Reference method	Internal standard	Extraction solvent	Sample preparation	Injection volume µL	Column T°	Detector	Stationary phase (SP) Mobile phase (MP)
8	In-house method	No	Methanol/H_2O (90:10)	Dissolve sample in 5mL of water. Fill to mark with methanol. Dilute with methanol. Filtrate though a 0.45 µm filter.	10	25	UV 280 nm	**SP**: Phenomenex Gemini C18, 150 x 2 mm; 3 µm; 0.2 mL/min **MP**: Methanol/H_2O acid formic acid 0.3%
9	In-house method	No	Acetonitrile	Sonicate for 20 mins. Filtrate through a 0.45 µm PTFE filter.	3	40	DAD 235 nm	**SP**: Zorbax ODS, 150 x 4,6 mm; 5 µm 1.0 mL/min **MP**: Acetonitrile/H_2O acid Phosphoric acid 0.1% (60:40, v/v)
10	Manufacturer's method	No	Mobile phase	Weight sample and add 4 mL Methanol + 6 mL H_2O buff / 25 mL mobile phase	5	NA	DAD 340 nm	**SP**: Zorbax Eclipse Plus C18, 150 x 4,6 mm; 5 µm; 0.3 mL/min **MP**: Methanol/ Buffer Na acetate/acetic acid (40:60, v/v)
11	In-house method	No	Acetonitrile	Dissolve the sample in 100 mL of acetonitrile. Sonicate for 10 minutes. Filtrate through a 0.45 µm PTFE filter.	10	40	DAD 300 nm	**SP**: Phenomenex Kromasil C18 100A 250 x 4.6 mm; 5 µm; 1.0 mL/min **MP**: Water /Acetonitrile/phosphoric acid 0.1% (60:40, v/v)
12	In-house method	No	Acetonitrile	Sonicate 15 Min. Filtrate 0.45 nylon filter.	10	35	DAD 254 nm	**SP**: Zorbax Eclipse C8, 150 x 4,6 mm; 5 µm 1.5 mL/min **MP**: Acetonitrile/H_2O acid Phosphoric acid 0.1%
14	CIPAC MT366	No	Methanol and Sodium acetate buffer 0.075N adjusted to pH6 with glacial acetic acid	Dissolve in 40mL of methanol and make up to 100mL with sodium acetate buffer. Sonicate for 5 min. Filtrate through 0.45 µm PTFE disk	10	40	DAD 340 nm	**SP**: Phenomenex Luna C18(2) 100A, 250 x 4.6 mm; 5 µm; 1.0 mL/min **MP**: Methanol/Sodium acetate buffer pH=6 (40:60)

to be followed

continues

ID Lab	Reference method	Internal standard	Extraction solvent	Sample preparation	Injection volume µL	Column T°	Detector	Stationary phase (SP) Mobile phase (MP)
15	CIPAC MT366	No	Methanol/sodium acetate buffer	Dilute with methanol and sodium acetate buffer (40:60 v/v); sonicate for 5 minutes; filtrate through 0,2 µm PP disk before injection	20	25	DAD 340 nm	**SP**: LiChrospher 100 RP18, 250 x 4 mm; 5 µm; 1.0 mL/min **MP**: Methanol/Sodium acetate buffer pH=6 (40:60)
16	In-house method	No	Water:acetonitrile (1:24)	Sonicate 10 min. Filtrate through Nylon 0.45 µm disk	5	30	VWD 230 nm	**SP**: Zorbax Eclipse XDB-C18, 250 x 4,6 mm; 5 µm; 1,1 mL/min **MP**: Acetonitrile/ 3.0% Acetonitrile in 0,1% H_3PO_4
17	In-house method	No	Acetonitrile	Ground and weight sample. Dilute in acetonitrile. Sonicate and make up to 250 mL	5	25	DAD 340 nm	**SP**: Kinetex C18 100A 100 x 4.6 mm; 2.6 µm; 5 mL/min **MP**: Acetonitrile/H_2O acid formic acid 0.1% (65:35, v/v)
20	CIPAC	No	Methanol / 20mM Na Acetate pH6 (with acetic acid)	Weight sample add 2mL methanol and 48mL methanol/20mM Na acetate pH6 (40/60). Sonicate for 30 min. Filtrate with 0.2 µm filter in vial	0.5	40	DAD 340 nm	**SP**: Kinetex Biphenyl 100 x 2.1 mm; 2.6 µm; 0.3 mL/min **MP**: Methanol/ Na Acetate 20 mM pH=6 acid ac. acetic
21	In-house method	No	Water pH=2 phosphoric acid	Sonicate for 1 minutes. Shake for 5 seconds. Filtrate through 0.45 µm nylon disk	2	40	UV 304 nm	**SP**: Phenomenex gemini NX C18 150 x 4.6 mm; 3 µm; 1.5 mL/min **MP**: Methanol/Acetonitrile/H_2O acid phosphoric acid pH = 2 (25:15:60, v/v/v)
22	In-house method *J. Chromatogr.* 1983;258:302-6	No	600 mL methanol + 400 mL 0.1 M acetic acid	NA	20	NA	UV 280nm	**SP**: Spherisorb S5 ODS 250 x 4.6 mm; 5 µm; 1.2 mL/min **MP**: Methanol/Acetic acid 0.1 M (60:40, v/v)

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APPENDIX A
The announcement letter

ANNOUNCEMENT/INVITATION
ITPT2022

Dear Colleagues,

We herewith cordially invite you to participate in the Italian Proficiency Test on the analysis of PPPs in SG, SC and EW. This exercise is organized by the Italian Laboratory of National Institute of Health – Department of Environment and Health. The ITPT2022 is scheduled to run from 15th January until 30th April 2022.

AIMS
Participation in proficiency tests is part of the QA/QC system of laboratories and provides them with an assessment of their analytical performance as well as a comparison with the performance of other laboratories. The general aim is to help laboratories demonstrate adequate analytical performance and, in case of underperformance, to help them identify sources of errors so that the necessary measures for quality improvement can be taken.

TEST ITEM
Ca. 10g of PPP test Item will be delivered to each participating lab.

TARGET ANALYTES
The analytes are:

Active Substance	Conc (g/100g)	Formulation
Spinosad	44.2	SC
Tau Fluvalinate	21.4	EW
Bentazone	87.0	SG

SHIPMENT AND RECEIPT OF THE TEST ITEM
The shipment of the Test Item is planned to start around 15th January 2022. If any laboratory will be on holiday in the week of the shipment, please inform the organizer to rearrange shipment.
Participants must check the integrity and condition of the materials upon receipt and to report within 48h if they accept the materials or not.

IMPORTANT DATES
- The shipment of the Test Items is planned to start around 15 January 2022.
- Submission of results and method information should be done by 30 April 2022.

PARTICIPATION FEE
The participation is free of charge.

RELEVANT DOCUMENTS
Participants are encouraged to employ the method typically run in their lab for these analytes.

SUPORT AND CONTACT INFORMATION
For any questions about the ITPT2022, please mail to angela.santilio@iss.it; valentina.picardo@iss.it
Best regards,
The ITPT2022 Organizing Team

APPENDIX B
Calendar and list of participants

CALENDAR for the ITPT2022

Activity	Dates
Opening of the ITPT2022	4th November 2021
Confirm the participation	30th November 2021
Shipment of the ITPT-PPP03 Test Item	15th January 2022
Confirmation of Sample Receipt and Acceptance	Within 48 h of receipt
Result Submission	30th January – 30th April 2022
Preliminary Report	May 2022
Final Report	June 2022

LIST OF PARTICIPANTS

Italian participants

Arianna Palchetti	*Laboratorio analisi alimenti e sicurezza dei prodotti – APPA BZ*
Luigi Bazzani Diego Tamoni Paola Rinaldi	*ARPA Emilia Romagna* *Sede secondaria laboratorio Multisito, sezione di Ferrara*
Leonardo Sabatino	*Ministero delle Politiche Agricole Alimentari e Forestali, Ispettorato centrale della tutela della qualità e repressione frodi dei prodotti agroalimentari - Laboratorio di Catania*
Tiziana Generali	*Istituto Superiore di Sanità, Roma*

European participants

Lajos Sándor Benke	*National Food Chain Safety Office Pesticide Analytical National Reference laboratory - Hungary*
Florentina Ciotea	*National Phytosanitary Authority - Romania*
Frantisek Csicsay	*Central Control and testing Institute in agriculture, ÚKSÚP - Slovakia*
Christoph Czerwenka	*AGES Group for contaminant and special analysis, Austrian Agency for Health and Food Safety - Austria*
Claudia Vinke	*Federal Office of Consumer Protection and Food Safety, Laboratory for formulation chemistry - Germany*
Cristina Ø Pedersen	*Laboratory for chemistry and microbiology, Danish Technological Institute - Denmark*
Isabelle Monisse	*AFSCA- Belgium*
Eva Jacobsen	*Danish Technological Institute - Aarhus, Denmark*
Kati hakala	*Finnish Food Authority - Finland*
Helen Karasali Anna Marousopoulou	*Laboratory of chemical Control of Pesticide, Benaki Phytopathological Institute –Greece*
Olga Nováková	*UKZUZ Central Institute for Supervising and Testing in Agriculture, National Reference laboratory, Department of testing Plant Protection Products – Czech Republic*
Benoit Saclier	*Service Commun des Laboratoires - France*
St. Nikolova Petar Ilchev	*CLCT, Bulgary*
Bernard de Rychel	*Walloon Agricultural Research Centre (CRA-W), Belgium*
Javier Garcìa-Hierro Navas	*Laboratorio Arbitral Agroalimentario, Spain*
Paul Martin-Carr Denis	*Pesticides Formulations Laboratory, DAFM, Ireland*

GLOSSARY

Active Ingredient. An Active Ingredient (AI) is the ingredient in a pharmaceutical drug or plant-health drug that is biologically active. Some products may contain more than one active ingredient.

Analyte. An analyte, component, or chemical species is a substance or chemical constituent that is of interest in an analytical procedure.

CAS number. A CAS Registry Number, also referred to as CASRN or CAS Number, is a unique numerical identifier assigned by the Chemical Abstracts Service (CAS) to every chemical substance described in the open scientific literature (currently including all substances described from 1957 through the present, plus some substances from the early or mid-1900s) including organic and inorganic compounds, minerals, isotopes, alloys and no structural materials (UVCBs, of unknown, variable composition, or biological origin). The registry maintained by CAS is an authoritative collection of disclosed chemical substance information. It currently identifies more than 141 million unique organic and inorganic substances and 67 million protein and DNA sequences, plus additional information about each substance. It is updated with around 15,000 additional new substances daily.

Chemical formula. A chemical formula is a way that chemists describe a molecule. The formula says what atoms, and how many of each type, are in the molecule. Sometimes the formula shows how the atoms are linked, and sometimes the formula shows how the atoms are arranged in space. The letter shows what chemical element each atom is.[1] The subscript shows the number of each type of atom.

% CV. The coefficient of variation (CV) is defined as the ratio of the standard deviation σ to the mean μ multiplied 100: $CV = (\sigma / \mu) \times 100$.

E isomer. It is the IUPAC convention of a molecular configuration, if the two groups of higher priority are on opposite sides of the double bond, the bond is assigned the configuration E (from the German word for "opposite" *entgegen*).

Grand Average. The grand mean or average is the mean of the means of several subsamples, as long as the subsamples have the same number of data points. For example, consider several lots, each containing several items. The items from each lot are sampled for a measure of some variable and the means of the measurements from each lot are computed. The mean of the measures from each lot constitutes the subsample mean. The mean of these subsample means is then the grand mean.

Homogeneity. Homogeneity and heterogeneity are concepts often used in the sciences and statistics relating to the uniformity in a substance or organism. A material or image that is homogeneous is uniform in composition or character (i.e., colour, shape, size, weight, height, distribution, texture, language, income, disease, temperature, radioactivity, architectural design, etc.); one that is heterogeneous is distinctly non uniform in one of these qualities.

Horwits Ratio. The Horwits ratio is a normalized performance parameter indicating the acceptability of methods of analysis with respect to among-laboratory precision (reproducibility).

Internal Standard. An internal standard in analytical chemistry is a chemical substance that is added in a constant amount to samples, the blank and calibration standards in a chemical analysis. This substance can then be used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration of the standards. This is done to correct for the loss of analyte during sample preparation or sample inlet. The internal standard is a compound that is very similar, but not identical to the chemical species of interest in the samples, as

the effects of sample preparation should, relative to the amount of each species, be the same for the signal from the internal standard as for the signal(s) from the species of interest in the ideal case.

MAD. In statistics, the Median Absolute Deviation (MAD) is a robust measure of the variability of a univariate sample of quantitative data. MAD = median of ($|X_i - \text{median (Xi)}|_{i=1,2...n}$).

Median. The median is the value separating the higher half of a data sample, a population, or a probability distribution, from the lower half. For a data set, it may be thought of as the "middle" value. For a continuous probability distribution, the median is the value such that a number is equally likely to fall above or below it. The median is a commonly used measure of the properties of a data set in statistics and probability theory. The basic advantage of the median in describing data compared to the mean (often simply described as the "average") is that it is not skewed so much by extremely large or small values, and so it may give a better idea of a "typical" value. Because of this, the median is of central importance in robust statistics.

Modified z-score. The z-score of an observation is defined as $Zi = (X - \mu) / \sigma$, where X is a sample, μ the sample mean and σ the standard deviation. In other words, data is given in units of how many standard deviations it is from the mean. Although it is common practice to use z-scores to identify possible outliers, this can be misleading in particularly for small sample sizes, so is better to use the modified z-score:

$$Zi = 0.6745 \text{ x } {}^{(Xi - \text{median})} / \text{MAD}$$

The modified z-scores with an absolute value of greater or lower than 3.5 be labelled as an outlier.

Outlier. An outlier is an observation that appears to deviate markedly from other observations in the sample. Identify potential outliers is important because it may indicate a bad data. For example, the data may have been coded incorrectly or an experiment may not have been run correctly. If it can be determined that an outlying point is in fact erroneous, then the outlying value should be deleted from the analysis (or corrected if possible). If it is not possible to simply delete the outlying observation, the use of robust statistical techniques may be considered.

Reference method. A reference method is an analytic procedure sufficiently free of random or systemic errors to make it useful for validating proposed new analytic procedures for the same analyte. This method has to be accuracy of a definitive method already certified demonstrated through direct comparison and must use primary reference material (standards, glasses, instruments). An in-house method it means that the method is not certified and made with the laboratory's instruments and techniques. The CIPAC methods is an analytical method make following CIPAC's instructions as the Manufacturer's method is make with the Manufacturer's instructions.

SD. The standard deviation (SD, also represented by the Greek letter sigma σ or the Latin letter s) is a measure that is used to quantify the amount of variation or dispersion of a set of data values. A low standard deviation indicates that the data points tend to be close to the mean of the set, while a high standard deviation indicates that the data points are spread out over a wider range of values.

Stability. The stability is a molecular characteristic of a chemical or compound; is the tendency of a material to resist change or decomposition in its natural environment or when exposed to air, heat, light, pressure or other natural conditions or due to internal reaction.

Z isomer. is the IUPAC convention of a molecular configuration, if the two groups of higher priority are on the same side of the double bond, the bond is assigned the configuration Z (from the German word for "together" *zusammen*).

Serie Rapporti ISTISAN
numero di dicembre 2022, 2° Suppl.

Stampato in proprio
Servizio Comunicazione Scientifica – Istituto Superiore di Sanità

Roma, dicembre 2022