Proficiency test for diquat and paraquat in soybean meal

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Wageningen, July 2020

WFSR report 2020.013

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Distribution list:
- Eighteen participating laboratories

Contents

Summary

In October 2019, a proficiency test for diquat and paraquat (bipyridylium herbicides/desiccants) in soybean meal was organised by Wageningen Food Safety Research (WFSR), part of Wageningen University & Research in accordance with ISO 17043. WFSR, is accredited for the organisation of proficiency tests in the field of contaminants, pesticides, mycotoxins, plant toxins and veterinary drugs in feed and feed ingredients according to ISO/IEC 17043 (R013). The primary goal of this proficiency test was to give participants the opportunity to evaluate and demonstrate their competence for the analysis of diquat and paraquat in soybean meal.

Two materials were prepared and dispatched on dry-ice to the participants. The consensus values of the two pesticides in each material are given in Table 1.

Table 1 *Consensus values of the diquat and paraquat in the proficiency materials.*

	Material A	Material B
Compound	Consensus value µg/kg	Consensus value µg/kg
diquat	267	96.7
paraquat	51.5	129

Material A and B were prepared by spiking a solution of diquat and paraquat to soybean meal to the required target concentrations. The materials were mixed with water followed by extensive mixing, freeze-drying and milling of the samples. Each participant received one test sample of each material.

Homogeneity assessment showed that both materials were sufficiently homogeneous for proficiency testing. The stability test demonstrated no statistically significant loss of the polar pesticides.

Eighteen laboratories participated in this proficiency test. The proficiency of the participants was assessed through z-scores, calculated using the consensus value and a target relative standard deviation of 25%.

The results of the proficiency test on polar pesticides in soybean meal are summarized in Table 2. A total of 72 z-scores could be calculated from the submitted results of which seven questionable z-scores and two unsatisfactory z-scores were reported. No false negative results were reported. Twelve participants achieved optimal performance for both materials by detecting both pesticides with the correct quantification, the absence of false negative results and reporting within the indicated deadline. Six participants reported questionable or unsatisfactory z-scores.

Table 2 *Summarized performance of laboratories reporting results in the proficiency test on diquat and paraquat in materials A and B.*

Compound	# of the results	Satisfactory performance (%)
	Material A (soybean meal)	
diquat	18	94
paraquat	18	94
	Material B (soybean meal)	
diquat	18	78
paraquat	18	83

Based on the results of this test it can be concluded that the variation in results for diquat in material A was higher than paraquat in material A and diquat and paraquat in material B. The interlaboratory reproducibility (RSD_R) ranged from 21 – 36%. The satisfactory results varied from 78 to 94%. No clear explanation could be found for the higher RSD_R for diquat (36%) in material A and the lower satisfactory results for diquat (78%) and paraquat (83%) in material B.

1 Introduction

Proficiency testing is conducted to provide participants with a powerful tool to evaluate and demonstrate the reliability of the data that are produced by the laboratory. Proficiency testing is an important requirement and demanded by ISO/IEC 17025:2017 [1].

The preparation of the materials, including the homogeneity and stability testing of the materials, and the evaluation of the quantitative results were carried out under accreditation according to ISO/IEC 17043:2010 [2] accreditation by the Dutch Accreditation Board (R013).

Diquat and paraquat are bipyridylium compounds that are used as herbicide or desiccant. Paraquat has been banned for use in the EU since 2007. Diquat was no longer approved in the EU from 2019 (max period of grace: 4 February 2020). Both compounds are however still used elsewhere. In the EU, maximum residue level(MRL) for diquat in soybeans is 0.3 mg/kg and for paraquat in soybeans is 0.02 mg/kg and has been set in (Regulation (EC) No 396/2005 [9] and amendments thereof. For the matrix soybean meal as such, no specific MRLs have been set and MRLs derived from soybeans after taking processing factors into account apply.

The aim of this proficiency test was to give participants the opportunity to evaluate or demonstrate their competence for the analysis of diquat and paraquat in soybean meal.

2 Material and methods

2.1 Scope of the proficiency test

This proficiency test (PT) focused on the pesticides diquat and paraquat in the feed matrix soybean meal. The target concentrations for the pesticides in this PT are presented in Table 3. The soybean meal is a by-product released during the extraction of soybean oil.

2.2 Material preparation

For preparation of the two PT materials A and B, soybean meal was used. Levels were artificially increased by spiking with diquat and paraquat. For each material, two kilograms were first fortified by adding a solution of a pesticide mix in water, aiming at the levels as presented in Table 3. The materials were mixed with approximately six litres of water, homogenized using an industrial mixer according to an in-house standard operating procedure [3]. The fortified slurries were freeze-dried, homogenized in a Stephan cutter, and stored in the freezer until use.

Table 3 *Target concentrations of polar pesticides in the proficiency materials.*

	Material A	Material B
Compound	**Target concentration (µg/kg)**	**Target concentration (µg/kg)**
diquat	300	100
paraquat	50	150

2.3 Sample identification

After homogenization, materials A and B were divided into sub-portions of approximately 50 grams in polypropylene, airtight closed containers of 125 ml. The containers were stored in the freezer until use.

The samples for the participants were randomly selected and coded using a web application designed for PTs (Annex 1). The code used was 2020/pesticides/soybean/000, in which the three digit number at the end of the code was automatically generated by the WFSR Laboratory Quality Services web application. One sample set was prepared for each laboratory consisting of one randomly selected sample of each material A and B. The codes of the samples for each sample set are presented in Annex 1. For homogeneity and stability testing, randomly selected containers of material A and B were used.

2.4 Homogeneity study

To verify the homogeneity of the PT materials, ten containers of material A and B were analysed in duplicate for diquat and paraquat. The homogeneity of the materials was assessed according to The International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [6] and ISO 13528:2015 [4]. For homogeneity a target standard deviation for proficiency assessment (σ_P) of 25% was used as a fit-for-purpose standard deviation, in line with the target RSD used in proficiency tests on pesticides as organised by the pesticides EURLs[10]. With this procedure the between-sample standard deviation (s_s) and the within-sample standard deviation (s_w) were compared with the standard deviation for proficiency assessment. The method applied for homogeneity testing is considered suitable if $s_w<0.5*\sigma_P$ and a material is considered adequately homogeneous if $s_s<0.3*\sigma_P$. The results of the homogeneity study, the grand mean with the corresponding RSD are presented in Table 4 and the statistical evaluation of material A and material B are presented in Annex 3. Diquat and paraquat in both materials fulfilled the homogeneity-criterion.

Table 4 *Concentration of pesticides in materials A and B obtained during homogeneity testing.*

Material code	Material A		Material B	
	Concentration µg/kg	RSD %	Concentration µg/kg	RSD %
diquat	282	8.17	98.4	6.18
paraquat	53.5	4.46	146	6.42

2.5 Stability of the materials

The stability of the mycotoxins in the PT materials was assessed according to [5, 7]. On January 20th, 2020, the day of distribution of the PT samples, six randomly selected containers of each material A and B were stored at <-18°C. Under these conditions it is assumed that diquat and paraquat are stable in the materials. Another six containers remained stored at <4 °C.

On April 13th, 2020, 87 days after distribution of the samples, for each of the storage conditions (< -18°C, <4 °C) six samples of materials A and B were analysed for both pesticides. For each set of test samples, the average of the results and the standard deviation were calculated.

It was determined whether a consequential instability of the analytes occurred [5, 7] in the materials stored at <4 °C. A consequential instability is observed when the average value of an analyte in the samples stored at <4 °C is more than $0.3\sigma_P$ below the average value of the analyte in the samples stored at <-18°C. If so, the instability has a significant influence on the calculated z-scores.

The results of the stability of materials A and B are presented in Annex 4. In none of the pesticide/storage condition combinations, a consequential difference was observed. Diquat and paraquat in the materials were therefore considered stable for the duration of the PT.

2.6 Interpretation of the results

A result was assigned as false negative result if a compound was '<[value]' or 'not detected' (nd), taking into account the reported scope of the participant, the consensus value and the reported LOQ for the compound by the participant. False negatives are indicated as 'FN'. False negatives are to be interpreted as unsatisfactory performance.

*For example: the consensus value of compound A is 70 µg/kg and the participant reported an LOQ= 20 µg/kg for this compound. Taken into account the 25% target standard deviation in this test, the -2z threshold would be at 35 µg/kg (70-(2*25% of 70)). Since the LOQ of this participant is lower than the -2z value, this participant should be able to detect the presence. If the LOQ would have been 40 µg/kg no false negative result would be assigned.*

Also, when no LOQ values were reported and the compound was reported as '<[value]' or (nd) a false negative result was assigned.

3 Organisational details

3.1 Participants

Nineteen participants registered for the participation in the PT and 18 participants reported their results. All participants were situated in Europe. One participant was unable to report result due to lack of laboratory capacity. Each participant was free to use their method of choice reflecting their routine procedures. The participants were asked to report the results through an existing web application designed for proficiency tests organised by WFSR.

3.2 Material distribution and instructions

Each participant received a randomly assigned laboratory code, generated by the web application. The sets of samples with the corresponding number, were sent to the PT participants on the 20th of January 2020. The sets of samples were packed in insulation boxes containing dry ice and were dispatched to the participants immediately by courier. The participants were asked to store the samples at <4 °C and to analyse the samples according to their routine practice. As reported by the participants, all parcels were received within 24 hours after dispatch, except one parcel that took 48 hours to reach the laboratory. All samples were received in good order.

The samples were accompanied by a letter describing the requested analysis (Annex 2) and an acknowledgement of receipt form. In addition, by e-mail, each participant received instructions on how to use the web application to report the results. Results should be reported as µg/kg product (no correction for moisture). Participants were asked to provide information on their analytical method (extraction solvent, clean-up procedure, internal standards used, detection technique, limit of detection, limit of quantification).

A single analysis result for both pesticides in each sample was requested. The deadline for submitting the quantitative results was the 2nd of March 2020, allowing the participants six weeks for analysis of the test samples. All results, were submitted within the deadline.

4 Statistical evaluation

The statistical evaluation was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [6], elaborated by ISO, IUPAC and AOAC and ISO 13528:2015 [4] in combination with the insights published by the Analytical Methods Committee [7,8] regarding robust statistics.

For the evaluation of the quantitative results, the consensus value, the uncertainty of the consensus value, the standard deviation for proficiency assessment and z-scores were calculated.

4.1 Calculation of the consensus value

The consensus value (X) was determined using robust statistics [4, 7, 8]. The advantage of robust statistics is that all values are taken into account: outlying observations are retained, but given less weight. Furthermore, it is not expected to receive normally distributed data in a proficiency test. When using robust statistics, the data do not have to be normally distributed in contrast to conventional outlier elimination methods.

The robust mean of the reported results of all participants, calculated from an iterative process that starts at the median of the reported results using a cut-off value depending on the number of results, was used as the consensus value [4, 7].

4.2 Calculation of the uncertainty of the consensus value

The uncertainty of the consensus value is calculated to determine the influence of this uncertainty on the evaluation of the participants. A high uncertainty of the consensus value will lead to a high uncertainty of the calculated participants z_a-scores. If the uncertainty of the consensus value and thus the uncertainty of the z_a-score is high, the evaluation could indicate unsatisfactory method performance without any cause within the laboratory. In other words, illegitimate conclusions could be drawn regarding the performance of the participating participants from the calculated z_a-scores if the uncertainty of the consensus value is not taken into account.

The uncertainty of the consensus value (the robust mean) is calculated from the estimation of the standard deviation of the consensus value and the number of values used for the calculation of the consensus value [4]:

$$u = 1.25 * \frac{\hat{\sigma}}{\sqrt{n}}$$

where:

u = Uncertainty of the consensus value;
n = Number of values used to calculate the consensus value;
$\hat{\sigma}$ = The estimate of the standard deviation of the consensus value resulting from robust statistics.

According to ISO 13528:2015 [4] the uncertainty of the consensus value (u) is negligible and therefore does not have to be included in the statistical evaluation if:

$u \leq 0.3\sigma_P$

where:
u = The uncertainty of the consensus value;
σ_P = Standard deviation for proficiency assessment (§3.3).

In case the uncertainty of the consensus value does not comply with this criterion, the uncertainty of the consensus value should be taken into account when evaluating the performance of the participants regarding the accuracy (§3.4). In case the uncertainty is > $0.7\sigma_P$ the calculated z-scores should not be used for evaluation of participants performance and are presented for information only.

4.3 Calculation of the standard deviation for proficiency assessment (σ_P)

A target standard deviation for proficiency assessment (σ_P) of 25% was used as a fit-for-purpose standard deviation which is in line with the target RSD used in proficiency tests on mycotoxins as organised by the EURL.

$\sigma_P = 0.25c$

where:
σ_P = Expected standard deviation in proficiency tests for animal feed;
c = Concentration of the analyte (µg/kg).

4.4 Performance characteristics with regard to the accuracy

For illustrating the performance of the participating participants with regard to the accuracy a z_a-score is calculated. For the evaluation of the performance of the participants, ISO 13528:2015 [4] is applied. According to these guidelines z_a-scores are classified as presented in Table 5.

Table 5 *Classification of z_a-scores.*

$\lvert z_a \rvert \leq 2$	Satisfactory
$2 < \lvert z_a \rvert < 3$	Questionable
$\lvert z_a \rvert \geq 3$	Unsatisfactory

If the calculated uncertainty of the consensus value complies with the criterion mentioned in §3.2, the uncertainty is negligible. In this case the accuracy z-score is calculated from:

$$z_a = \frac{\bar{x} - X}{\sigma_P} \quad \text{Equation I}$$

where:
z_a = Accuracy z-score;
$\bar{x}$ = The average result of the laboratory;
X = Consensus value;
σ_P = Standard deviation for proficiency assessment.

However, if the uncertainty of the consensus value does not comply with the criterion mentioned in §3.2, it could influence the evaluation of the participants. Although, according to ISO 13528 in this case no z-scores can be calculated, we feel that evaluation of the participating participants is of main importance justifying the participating participants' effort. Therefore in this case, the uncertainty is taken into account by calculating the accuracy z-score [4]:

$$z'_a = \frac{\bar{x} - X}{\sqrt{\sigma_P^2 + u^2}} \qquad \text{Equation II}$$

where:

z'_a = Accuracy z-score taking into account the uncertainty of the consensus value;
$\bar{x}$ = The average result of the laboratory;
X = Consensus value;
σ_P = Standard deviation for proficiency assessment;
u = Uncertainty of the consensus value.

A consequential instability of the proficiency materials can influence the evaluation of the laboratory performance. Therefore, in that case the consequential instability is taken into account when calculating z-scores. Because instability only regards one side of the confidence interval (a decrease of the concentration) this correction only applies to the lower 2s limit and results in an asymmetrical confidence interval.

In the case of a consequential instability the accuracy z-score for the participants that reported an amount below the consensus value is corrected for this instability by:

$$z_{ai} = \frac{\bar{x} - X}{\sqrt{\sigma_P^2 + \Delta^2}} \qquad \text{Equation III}$$

where:

z_{ai} = Accuracy z-score taking into account the instability of the consensus value;
$\bar{x}$ = The average result of the laboratory;
X = Consensus value;
σ_P = Standard deviation for proficiency assessment;
Δ = Difference between average concentration of compound stored at <-18 °C and average concentration at <4 °C.

In some cases the uncertainty of the consensus value does not comply with the criterion in §3.2 and a consequential instability is observed. In this case the z'_a-score for the participants that reported an amount below the consensus value is corrected for this instability by:

$$z'_{ai} = \frac{\bar{x} - X}{\sqrt{\sigma_P^2 + \Delta^2 + u^2}} \qquad \text{Equation IV}$$

where:

z'_{ai} = Accuracy z-score taking into account the uncertainty and instability of the consensus value;
$\bar{x}$ = The average result of the laboratory;
X = Consensus value;
σ_P = Standard deviation for proficiency assessment;
Δ = Difference between average concentration of compound stored at <-18 °C and average concentration at <4 °C;
u = Uncertainty of the consensus value.

5 Methods and results

5.1 Participants

Nineteen participants registered for the PT and 18 participants submitted their results. Each participant was free to use their method of choice reflecting their routine procedures. The performance of individual participants is summarized in Annex 7.

5.2 Methods of analysis applied by participants

An overview of the information provided by the participants regarding the methods applied in this PT is presented in Annex 5. The information provided was not always complete. Four participants provided no information at all.

In general, diquat and paraquat were extracted under (strong) acidic conditions (water/methanol with HCl or formic acid). Four participants performed the extraction at higher temperature (80°C). In most cases no clean-up was used, some laboratories used an SPE clean-up. In most cases, isotopic labelled diquat and paraquat were used as internal standards. The extracts were analysed by LC-MS/MS in all cases.

Ranges for the reported limits of detection (LODs) and limits of quantification (LOQs) for the pesticides are presented in Table 6. One participant reported an LOQ of 0.01 µg/kg which may have been an unit error. Four participants did not indicate the LODs and LOQs of the method used.

Table 6 *Overview of reported LOD and LOQ reported by the participants.*

Compound	LOD (µg/kg)	LOQ (µg/kg)
Diquat	1.7 - 10	5.6 - 20
Paraquat	2 - 19	6.8 - 20

5.3 Performance

The quantitative performance was assessed through z-scores. The individual z-scores obtained by each participant, including their graphical representation, for pesticides in materials A and B are summarised in Annex 6. A summary of the performance of the participants in this PT is provided in Annex 7.

A summary of the statistical evaluation of the PT results is presented in Table 7. This table include all relevant parameters: the consensus value (CV), the uncertainty of the assigned value (u), the standard deviation for proficiency assessment (σ_p) and the robust (relative) standard deviation, based on participants' results.

For paraquat in material A the uncertainty of the consensus value did comply with the criterion $u \leq 0.3\sigma_p$ and was therefore considered as negligible. The uncertainty of the consensus value (u) in material A exceeded $0.3\sigma_p$ for diquat and in material B for paraquat and diquat, and therefore, the uncertainty of the consensus value was taken into account in the evaluation of the z-scores. For material A, one of the reported results for diquat was a questionable result (PT9312), and one of the reported results for paraquat was questionable (PT9370). For material B, three of the reported results for diquat were questionable (PT9367, PT9378, PT9380) and one result was unsatisfactory (PT9312) and for paraquat two of the reported results were questionable (PT9376, PT9380) and one result was unsatisfactory (PT9312).

Table 7 *Parameters of diquat and paraquat in material A.*

	Material A		Material B	
	diquat	paraquat	diquat	paraquat
CV (μg/kg)	267	51.5	96.7	129
Lowest concentration (μg/kg)	56	32	10	30.2
Highest concentration (μg/kg)	410	85	160	352
u (μg/kg)	28.5	3.14	8.01	10.5
σ_p (μg/kg) (25%)	66.6	12.9	24.2	32.1
$u>0.3\sigma_p$	Yes	No	Yes	Yes
robust σ (μg/kg)	96.6	10.6	27.2	35.8
robust σ (%) (RSD_R)	36.2	20.6	28.1	27.8
# reported	18	18	18	18
# quantitative results	18	18	18	18
$\|z\|\leq 2$	17	17	14	15
$2<\|z\|<3$	1	1	3	2
$\|z\|\geq 3$			1	1
s z-scores (%)	94	94	78	83

The consensus values for diquat and paraquat in material A were respectively 267 and 51.5 μg/kg and in material B respectively 96.7 and 129 μg/kg.

The robust relative standard deviation (RSD_R) was calculated according to ISO13528:2015 [4] for informative purposes only. In this study it was used as a good estimation of the interlaboratory variability. The RSD_R values for each pesticide in both materials are shown in Annex 6 and in Table 7.

For material A, the robust standard deviations (RSD_R) of the reported results for paraquat (21%) in A was below the target standard deviation (25%) and for diquat (36%) the RSD_R exceeded the target standard deviation. For material B, the RSD_R values were close to the target standard deviation for both pesticides (28%).

For material A, 94% of the results were rated with satisfactory z-scores ($|z|\leq 2$), 6% of the results felt into the questionable range with $2<|z|<3$. For material B 81% of the results were rated with satisfactory z-scores ($|z|\leq 2$), 14% of the results felt into the questionable range with $2<|z|<3$ and 6% of the results felt into the unsatisfactory range with $|z|\geq 3$.

In Annex 7 an overview of the overall performance of each participant in this PT is summarised. For the two materials combined, a maximum of 4 satisfactory z-scores could be obtained, and ‘4 out of 4’ therefore reflects an optimal performance in terms of scope and capability for quantitative determination.

All 18 participants analysed the materials for both pesticides. Out of these 18 participants, 12 participants achieved optimal performance for both materials by detecting both pesticides with correct quantification, the absence of false positive and/or false negative results, and reporting all results within the set deadline.

6 Discussion and conclusions

Eighteen participants participated in the proficiency test on diquat and paraquat in the feed matrix soybean meal.

Two materials were sent to the participants. The pesticides were homogeneously distributed in the materials. An overview of each participant's performance is shown in Annex 7 and a summary of the results is presented in Table 6.

Out of 18 participants 12 showed optimal performance by detecting both pesticides with a correct quantification, the absence of false positive or false negative results and reporting within the deadline. Six participants reported questionable or unsatisfactory z-scores. A total of seven questionable z-scores and two unsatisfactory z-score were reported.

For the pesticides in material A, 94% of the reported results were satisfactory. In material B, 78% of the reported results were satisfactory for diquat and 83% for paraquat.

The robust relative standard deviation (RSD_R) was close to the target standard deviation, with the exception of diquat in material A (36%), indicating that the 25% target standard deviation is reasonably reflecting the current interlaboratory variability for diquat and paraquat in soybean meal.

Overall, for diquat and paraquat in both materials combined, 87.5% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 9.7% of the results fell into the questionable range with $2<|z|<3$ and 2.8% of the results fell into the unsatisfactory range with $|z| \geq 3$.

Based on the results of this proficiency test it was concluded that:

- The satisfactory results for diquat and paraquat varied from 78-94% in this proficiency test. No explanation can be given for the lower satisfactory results for diquat (78%) and paraquat (83%) in material B as opposed to material A.
- The interlaboratory reproducibility (RSDR) ranged from 21 – 36%.
- Overall result of this PT: 67% of the participants showed optimal performance.

References

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9 Regulation (EC) No 396/2005 of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council 91/414/EEC.

10 Target standard deviation for proficiency assessment (σ_P) of 25% used in proficiency tests on pesticides organised by the pesticides EURLs[10]. https://www.eurl-pesticides.eu/library/docs/allcrl/EUPT-General_Protocol_V9_2020.pdf

Annex 1 Codification of the samples

Participants code	Last three digits of codes Material A*	Last three digits of codes Material B*
PT9312	316	419
PT9367	205	837
PT9368	431	995
PT9369	561	352
PT9370	181	959
PT9371	321	400
PT9372	552	916
PT9373	468	516
PT9374	987	515
PT9375	847	104
PT9376	269	463
PT9377	703	733
PT9378	224	169
PT9379	902	220
PT9380	386	724
PT9381	426	583
PT9382	575	137
PT9383	344	110
PT9384	793	929

* All sample codes start with 2020/pesticides/soybean/

Annex 2 Instruction letter

WAGENINGEN
UNIVERSITY & RESEARCH

P.O. Box 230 | 6700 AE WAGENINGEN | The Netherlands

Dear Madam/Sir,

Thank you very much for your interest in the proficiency test for the analysis of the polar pesticides diquat and paraquat in the feed matrix soybean meal. Hereby I send you a parcel containing two randomly coded samples. Each sample consists of approximately 50 grams of test material.

Please fill out the accompanying acknowledgement of receipt form and return it immediately upon receipt of the samples, preferably by e-mail (pt.wfsr@wur.nl)

Instructions:

- After arrival the samples should be stored at +4 °C.
- Before analysis, homogenize them according to your laboratory's procedure.
- Treat the test material as if it was a sample for routine analysis. Report one result and not an average of multiple measurements for each sample.
- Report all results in **µg/kg** for the product as received (i.e. no correction for moisture content is needed). When a pesticide is not within your scope, please report 'nt' (not tested) in the web application. Do not use the option 'detected' from the web application. When a pesticide is 'not detected' or the result is below your LOQ, report the result as <LOQ-value and specify the value (e.g. <20 µg/kg).
- Please use the web application for entering your results (https://crlwebshop.wur.nl/apex/f?p=307:LOGIN). Information about the use of this web application was sent to you earlier by e-mail.
- The deadline for submitting test-results for this test is **2th of March 2020.**

100years
1918 — 2018

Wageningen Food Safety Research

DATE
January 20, 2020

SUBJECT
Instruction proficiency test polar pesticides diquat and paraquat in feed matrix soybean meal.

OUR REFERENCE
2000730/WFSR

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January 20, 2020

OUR REFERENCE
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PAGE
2 of 2

- Your username is:
- Your password is:
- Your lab code to enter this proficiency test is:

- Please inform us about your applied method and detection technique (via the web application).

Please contact me if you have any questions or need any assistance.

With kind regards,

Diana Pereboom
Proficiency tests

pt.wfsr@wur.nl

Annex 3 Statistical evaluation of homogeneity data

Sample No.	Diquat in material A (µg/kg) Replicate 1	Replicate 2
Hom/A001	265	265
Hom/A002	252	311
Hom/A003	263	288
Hom/A004	315	260
Hom/A005	289	288
Hom/A006	252	307
Hom/A007	310	259
Hom/A008	285	317
Hom/A009	303	276
Hom/A010	290	249
Grand mean	282	
Cochran's test		
C	0.219	
C_{crit}	0.602	
$C < C_{crit}$?	NO OUTLIERS	
Target $s = \sigma_P$	70.5	
s_x	10.4	
s_w	28.9	
s_s	0.000	
Critical= 0.3 σ_P	21.2	
s_s < critical?	ACCEPTED	
$s_w < 0.5\ \sigma_P$?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

Sample No.	Paraquat in material A (µg/kg) Replicate 1	Replicate 2
Hom/A001	54.6	54.5
Hom/A002	50.3	54.1
Hom/A003	52.2	57.8
Hom/A004	56.8	52.9
Hom/A005	54.2	53.4
Hom/A006	49.0	49.3
Hom/A007	54.3	54.3
Hom/A008	52.6	52.7
Hom/A009	52.9	53.3
Hom/A010	58.2	53.2
Grand mean	53.5	
Cochran's test		
C	0.369	
C_{crit}	0.602	
$C < C_{crit}$?	NO OUTLIERS	
Target $s = \sigma_P$	13.4	
s_x	1.89	
s_w	2.09	
s_s	1.17	
Critical= 0.3 σ_P	4.02	
s_s < critical?	ACCEPTED	
$s_w < 0.5\ \sigma_P$?	ACCEPTED	

Sample No.	Diquat in material B (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	99.4	93.3
Hom/A002	112	97.4
Hom/A003	96.0	90.8
Hom/A004	104	99.9
Hom/A005	99.1	102
Hom/A006	88.3	96.5
Hom/A007	89.6	102
Hom/A008	100	107
Hom/A009	92.9	105
Hom/A010	102	92.0
Grand mean	98.4	
Cochran's test		
C	0.256	
C_{crit}	0.602	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	24.6	
s_x	4.10	
s_w	6.33	
s_s	0.000	
Critical= 0.3 σ_P	7.38	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

Sample No.	Paraquat in material B (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	152	140
Hom/A002	150	135
Hom/A003	153	155
Hom/A004	144	138
Hom/A005	146	166
Hom/A006	142	138
Hom/A007	170	136
Hom/A008	136	145
Hom/A009	146	144
Hom/A010	147	143
Grand mean	98.4	
Cochran's test		
C	0.550	
C_{crit}	0.602	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	36.6	
s_x	5.92	
s_w	10.2	
s_s	0.000	
Critical= 0.3 σ_P	11.0	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

Annex 4 Statistical evaluation of stability data

*Statistical evaluation for **diquat in material A.***

Storage temperature	<-18 °C	<4 °C
Time (days)	0	89
Calculated amounts (µg/kg)	*	311
	336	320
	298	292
	287	322
	327	303
	302	340
Average amount (µg/kg)	310	314
n	5	6
st. dev (µg/kg)	20.6	16.7
Difference		-4.31
$0.3*\sigma_P$		23.3
Consequential difference? Diff < $0.3*\sigma_P$		No

*Outlier according to grubbs'test

*Statistical evaluation for **paraquat in material A.***

Storage temperature	<-18 °C	<4 °C
Time (days)	0	89
Calculated amounts (µg/kg)	*	57.1
	58.6	56.0
	52.1	60.4
	58.5	58.5
	55.7	52.3
	59.0	59.5
Average amount (µg/kg)	56.8	57.3
n	5	6
st. dev (µg/kg)	2.92	2.91
Difference		-0.51
$0.3*\sigma_P$		4.26
Consequential difference? Diff < $0.3*\sigma_P$		No

*Outlier according to grubbs'test

*Statistical evaluation for **diquat in material B.***

Storage temperature	<-18 °C	<4 °C
Time (days)	0	89
Calculated amounts (µg/kg)	93.6	78.0
	100	108.7
	102	85.2
	84.0	92.4
	98.1	97.8
	97.6	114.5
Average amount (µg/kg)	95.9	96.1
n	6	6
st. dev (µg/kg)	6.45	13.9
Difference		-0.22
0.3*σ_P		7.19
Consequential difference? Diff < 0.3*σ_P		No

*Statistical evaluation for **paraquat in material B.***

Storage temperature	<-18 °C	<4 °C
Time (days)	0	89
Calculated amounts (µg/kg)	167	158
	169	183
	187	200
	173	188
	182	184
	177	170
Average amount (µg/kg)	176	181
n	6	6
st. dev (µg/kg)	7.56	14.6
Difference		-4.74
0.3*σ_P		13.2
Consequential difference? Diff < 0.3*σ_P	No	No

Annex 5 Overview of the applied methods

Lab	Sample purification	Internal standard	LOD µg/kg		LOQ µg/kg		Detection method
			Diquat	Paraquat	Diquat	Paraquat	
PT9312	Water/formic acid extraction plus cleanup with Bond Elut C18	Diquat-D4, Paraquat-D6	3	6	10	20	LC-MS/MS
PT9367	Acid hydrolysis and clean-up	Yes	5	5	10	10	LC-MS/MS
PT9368	2.5g sample + 25ml MeOH/HCl 0.1M after centrifugation fill a vial	Paraquat-D6	5	5	10	10	LC-MS/MS
PT9369	Extraction with an acidified mixture of methanol and water	Diquat-D4, Paraquat-D8			10	10	LC-MS/MS
PT9370	1:1 mixture of methanol + aqueous HCl 0.1M, extraction in shaking water bath at 80 °C for 15 minutes, freeze- out and cold centrifugation.	Diquat-D4, Paraquat-D8	5	5	10	10	LC-MS/MS
PT9371							
PT9372	0.5M HCL in 40% MeOH		10	10	10	10	LC-MS/MS
PT9373			10	10			LC-MS/MS
PT9374							LC-MS/MS
PT9375	Quick Polar Pesticides-Method PO V10.1; 5.2.3.Extraction B) Procedure for Paraquat and Diquat	Diquat-D4, Paraquat-D6			20	20	LC-MS/MS
PT9376	weigh 5 gram, add internal standard, add 20 ml water, vortex, add 20 ml extraction solvent (50% methanol, 0.15% HCl), vortex, place in water bath (80 degrees) for 20 minutes, cool down, centrifuge @ 3600rpm for 5 minutes, place in filter vial	Diquat-D6, Paraquat-D8			10	20	LC-MS/MS
PT9377	Weigh 1 g of the sample into a tube (50 mL). Add 10 mL of MeOH+3% formic acid	Paraquat-D6			10	10	LC-MS/MS
PT9378	Diquat: 5g of matrix, 10mL of Methanol, heat until 80 ºC, cool down, centrifuge and filter. Paraquat: 5g of matrix, 5g of water, 10mL of Methanol 3% formic acid, heat until 80 ºC, cool down, centrifugue and filter.				10	10	LC-MS/MS
PT9380	2g sample+(9mL water+1mL EDTA 10% solution)+10mL(MeOH/HCl 0.1M 1:1 solution)+ 80 °C 15 minutes	Not used					LC-MS/MS
PT9381	In accordance with Quick polar Pesticides method (QuPPe) and QuPPe-AO with following exceptions: extraction with H2O:MeOH:Formic acid = 60:39:1 instead of 49.5:49.5:1. After heating, extraction with DCM for better separation	Diquat-D4, Paraquat-D6	17	19	20	20	LC-MS/MS
PT9382	Acidified extraction and SPE.	Isotopic labeled standards	1.7	2	5.6	6.8	LC-MS/MS
PT9383					0.01	0.01	
PT9384	Quick Polar pesticides method						LC-MS/MS

Annex 6 Results material A and B

Lab code	Material A diquat CV: 267 µg/kg u: 28.5 µg/kg σ_p: 66.6 µg/kg robust σ: 96.6 µg/kg (36%)		Material A paraquat CV: 51.5 µg/kg u: 3.14 µg/kg σ_p: 12.9 µg/kg robust σ: 10.6 µg/kg (21%)		Material B diquat CV: 96.7 µg/kg u: 8.01 µg/kg σ_p: 24.2 µg/kg robust σ: 27.2 µg/kg (28%)		Material B paraquat CV: 129 µg/kg u: 10.5 µg/kg σ_p: 32.1 µg/kg robust σ: 35.8 µg/kg (28%)	
	Result (µg/kg)	z'_a-score	Result (µg/kg)	z_a-score	Result (µg/kg)	z'_a-score	Result (µg/kg)	z'_a-score
PT9312	56	**-2.91**	54	0.20	10	**-3.40**	352	**6.61**
PT9367	410	1.98	43	-0.66	160	**2.49**	118	-0.31
PT9368	370	1.43	57	0.43	105	0.33	127	-0.05
PT9369	206.6	-0.83	42.2	-0.72	90.8	-0.23	119.4	-0.27
PT9370	230	-0.50	85	**2.61**	90	-0.26	110	-0.55
PT9371	318	0.71	43	-0.66	110	0.52	145	0.49
PT9372	344	1.07	53	0.12	121	0.96	131	0.07
PT9373	263	-0.05	62	0.82	91	-0.22	173	1.32
PT9374	290	0.32	48	-0.27	130	1.31	120	-0.25
PT9375	311	0.61	51	-0.04	110	0.52	149	0.61
PT9376	293	0.37	64	0.97	125	1.11	213	**2.50**
PT9377	187	-1.10	32	-1.51	62	-1.36	79	-1.46
PT9378	130	-1.88	56	0.35	45	**-2.03**	100	-0.84
PT9380	143.5	-1.70	60.3	0.69	38.7	**-2.28**	30.2	**-2.91**
PT9381	370	1.43	47	-0.35	98	0.05	150	0.64
PT9382	292.7	0.36	59.1	0.59	108.2	0.45	153.1	0.73
PT9383	307	0.56	48	-0.27	101	0.17	128	-0.02
PT9384	184	-1.14	35.3	-1.26	66.2	-1.20	62.4	-1.96

C = consensus value (robust mean)

u = uncertainty of consensus value

σ_p = target standard deviation for proficiency

robust σ = robust (relative) standard deviation based on participants' results

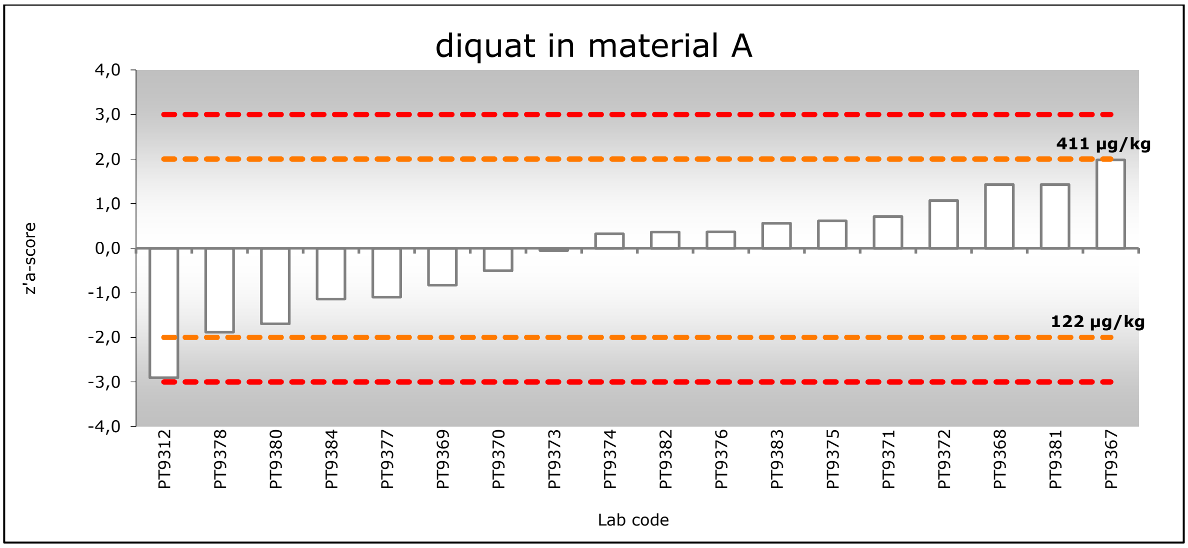

Figure a *Graphical representation of the z'$_a$-scores for diquat in material A. The X ± 2σ$_P$ lines (dotted) are calculated according to equation II in §3.4.*

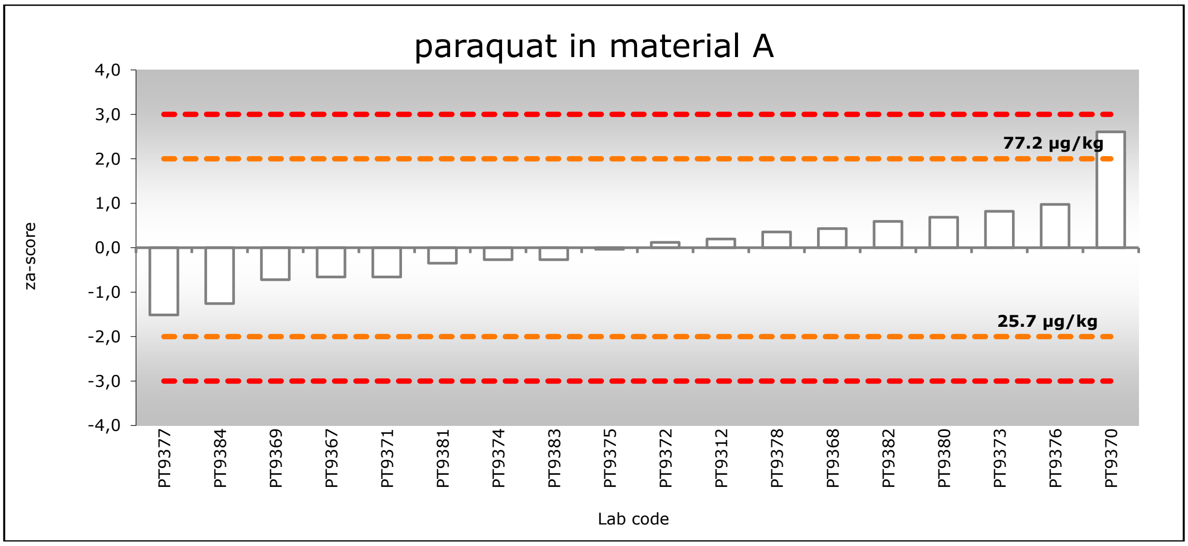

Figure b *Graphical representation of the z$_a$-scores for paraquat in material A. The X ± 2σ$_P$ lines (dotted) are calculated according to equation I in §3.4.*

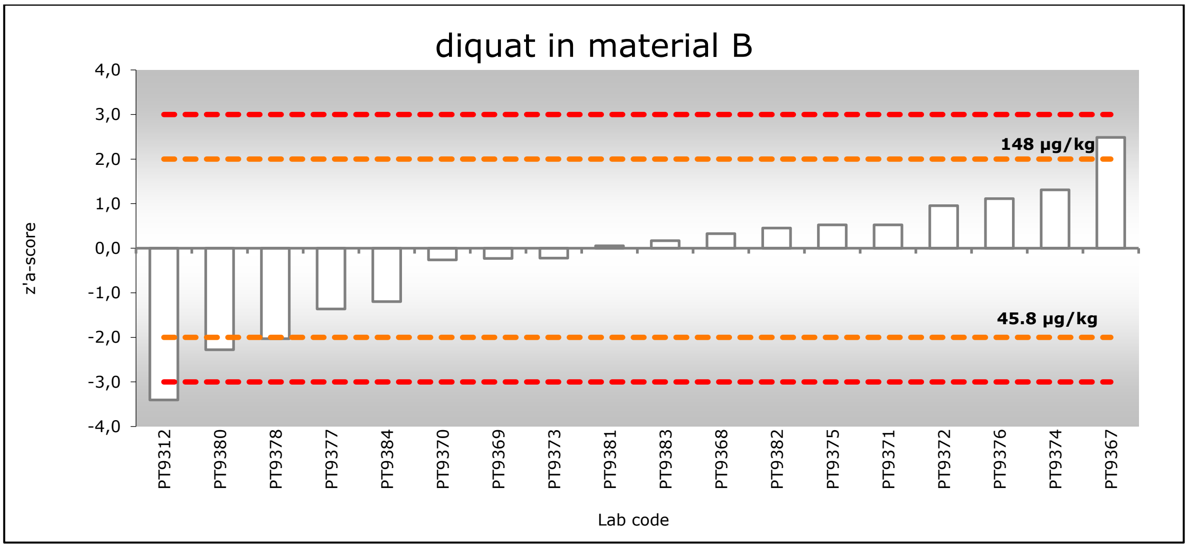

Figure c *Graphical representation of the z'$_a$-scores for diquat in material B. The X ± 2σ_P lines (dotted) are calculated according to equation II in §3.4.*

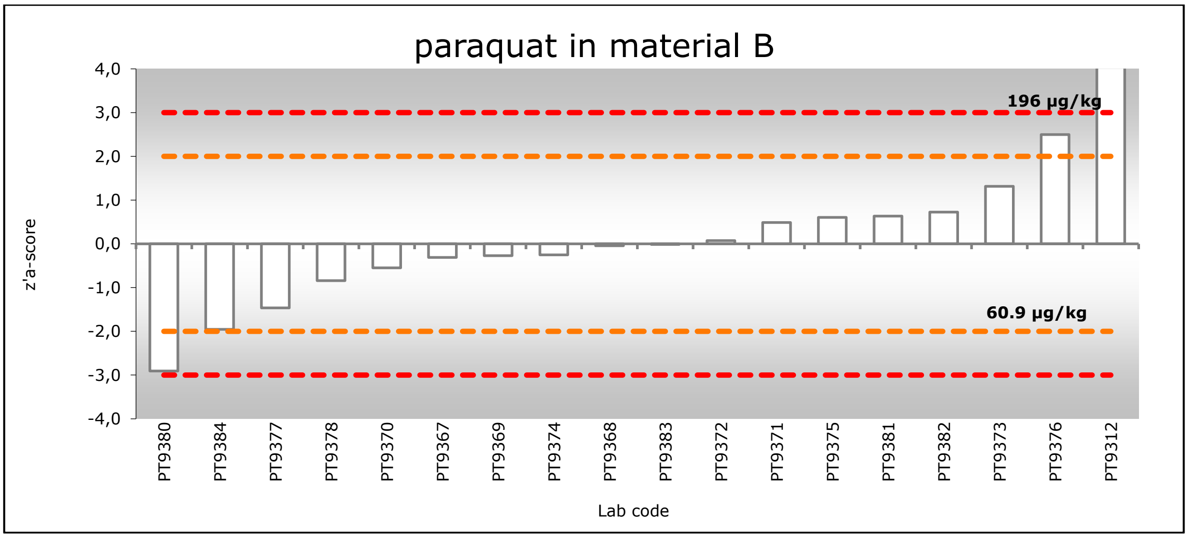

Figure d *Graphical representation of the z'$_a$-scores for paraquat in material B. The X ± 2σ_P lines (dotted) are calculated according to equation II in §3.4.*

Annex 7 Overview performance per laboratory

Laboratory code	Satisfactory performance
PT9312	1 of 4
PT9367	3 of 4
PT9368	4 of 4, **optimal performance**
PT9369	4 of 4, **optimal performance**
PT9370	3 of 4
PT9371	4 of 4, **optimal performance**
PT9372	4 of 4, **optimal performance**
PT9373	4 of 4, **optimal performance**
PT9374	4 of 4, **optimal performance**
PT9375	4 of 4, **optimal performance**
PT9376	3 of 4
PT9377	4 of 4, **optimal performance**
PT9378	3 of 4
PT9380	2 of 4
PT9381	4 of 4, **optimal performance**
PT9382	4 of 4, **optimal performance**
PT9383	4 of 4, **optimal performance**
PT9384	4 of 4, **optimal performance**

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