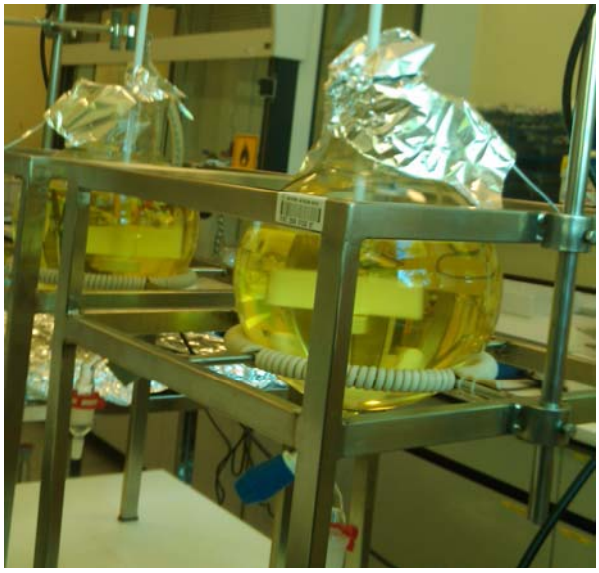
JRC Scientific and Technical Reports

Report of an interlaboratory comparison organised by the EU Reference Laboratory for Food Contact Materials

ILC01 2009 - DIDP in Oil

Laboratory performance and precision criteria of a harmonised method

S. Bratinova, M. Multari, S. Spalla and C. Simoneau



EUR 24387 EN 2010

SANCO/2008/FOODSAFETY/0047-Contact Materials

JRC EUROPEAN COMMISSION

ihcp Institute for Health and Consumer Protection

The mission of the JRC-IHCP is to protect the interests and health of the consumer in the framework of EU legislation on chemicals, food, and consumer products by providing scientific and technical support including risk-benefit assessment and analysis of traceability.

European Commission
Joint Research Centre
Institute for Health and Consumer Protection

Contact information
Address: DG JRC, IHCP TP 260, I-21020 Ispra
E-mail: catherine.simoneau@jrc.ec.europa.eu
Tel.: 39.0332.785889
Fax: 39.0332.785707
http://crl-fcm.jrc.ec.europa.eu/
http://ihcp.jrc.ec.europa.eu/
http://www.jrc.ec.europa.eu/

JRC58357
EUR 24387 EN
ISBN 978-92-79-15867-4
ISSN 1018-5593
doi:10.2788/97370

Luxembourg: Publications Office of the European Union

Printed in Italy

Report of the interlaboratory comparison

DIDP in Oil

EC-JRC-IHCP
November 2009

Stefanka Bratinova, Monica Multari, Stefano Spalla and Catherine Simoneau

Table of content

1. Summary

The Institute for Health and Consumer Protection (IHCP) of the European Commission's Directorate-General Joint Research Centre hosts the European Union Reference Laboratory for Food Contact Materials (EURL-FCM). One of its core tasks is to organize interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs). This report presents the results of the second ILC of the EURL-FCM which focused on the determination of Di-isodecyl phthalate (DIDP) in an oil matrix. The aim was to develop and perform the validation of a method for the analysis of DIDP (as model substance for a technical mixture of phthalates) from oil (as simulant for fatty foods). The strategy rose from the proficiency tests on plasticisers conducted by the EURL-FCM for the NRLs in 2008 (both in gaskets and in oil) that highlighted that the substances of lesser performance were technical mixtures of phthalates (DINP, DIDP) and it was decided to thus strategically deploy a follow up work item for 2009 with the development and validation of an improved method.

The test material used in this exercise was an industrial source of sunflower oil which was spiked with several levels of DIDP by the EURL-FCM. The EURL completed a preparation phase and distributed several concentration levels of spiked solvent and oil samples for the analysis of DIDP. This exercise was used both as proficiency testing and to validate a standard operating procedure (SOP) for the determination of DIDP in oil that was written by the EURL based on the most performant methods used by NRLs in the proficiency test of 2008. The homogeneity and stability studies were performed by the EURL-FCM laboratory. Testing of the developed SOP was performed by the EURL and a final SOP sent to NRLs with the relevant documents and results templates for reporting. Lots of fortified oils were sent to the NRLs. A two months completion period was established. Participation of local laboratories under NRLs was encouraged (by producing 60 samples). There were 28 participants to whom samples were dispatched 24 of which submitted results. From the EURL-NRL network 23 laboratories out of 24 reported results. There were 2 guests from Germany that provided results as well. Participants were invited to report four replicates measurements under repeatability conditions. The ILC was closed permanently in the middle of October for statistical interpretation.

Based on the results in this precision experiment the method performance was assessed through evaluation of the repeatability and reproducibility standard deviation (SD) according to the mechanism described in ISO 5725 [11,12]. The assigned value and its uncertainty were obtained as a consensus values after applying the robust statistics to the results obtained from the participants. Laboratory results were rated with z and z' scores in accordance with ISO 13528 [1]. Standard deviations for proficiency assessment (also called target standard deviations) were set based on Horwitz equation. The participation of the laboratories was regarded as satisfactory for the aim of the precision experiment with regards of the numbers of received results thanks to the proactive involvement of the NRLs-FCM. As a conclusion for participation and laboratory performance, this ILC showed: A noted increase in participation compared to the similar exercise of 2008. The number of laboratories submitting results for DIDP in oil rose from 17 to 25. This was due in part from the experience acquired in the previous year exercise as well as to the provision by the EURL of both the method description in a CEN like format as

well as of the internal standard.
A great increase in laboratory performance compared to 2008 with 76-92% of successful achievement of results from the participants within the tolerance limits (range 76-92% depended on concentration level considered) compared to 59% in 2008. In particular the performance at the concentration level of the SML was 80% compared to 59% for the same exercise in 2008. The harmonisation of the procedure and following a harmonised method for determination of DIDP in oil in 2009 resulted in a decrease more then 2.5 times in the reproducibility SD from 37% to 14 % for the concentration level around SML of 9 mg/kg while the repeatability SD remained almost the same – 6.5%.

2. Introduction

Interlaboratory comparison (ILC) studies are an essential and very important element of laboratory quality assurance, which allow individual laboratories to compare their analytical results with those from other laboratories while providing them objective standards to perform against.

One of the core duties of the European Union Reference Laboratories is to organise ILCs, as is stipulated in Regulation (EC) No 882/2004 of the European Parliament and of the Council [6].

In accordance with the above requirements the European Union Reference Laboratory for Food Contact Material (EURL-FCM) organised in 2009 for the second year several ILCs for the network of appointed National Reference Laboratories (NRLs).

The scopes of the ILC tests for 2009 were discussed and agreed on the plenary meeting with all NRLs held in December 2008 at JRC, Ispra, Italy. During that meeting one preference was expressed for a follow up of the 2008 ILC on phthalate in oil and the decision was made in consensus that for 2009 ILCs would be organised by the EURL-FCM with an aim towards the validation of a method preliminary agreed upon and drafted by the EURL-FCM as standard operating procedure (SOP).

The first phase of the ILC consisted of the collection of methods that were used in the 2008 exercise, based on a questionnaire sent and collected from NRLs.

The second phase consisted for the EURL in drafting a SOP based on the answers to the questionnaire and taking into consideration the performance of the laboratories.

In the third phase the NRLs received the samples (solvent, DIDP in solvent at 3 different levels, fortified DIDP in oil at 3 different levels) and analysed them, according to the SOP from the second phase.

The final phase was the statistical treatment of the results reported by the laboratories results and their presentation in the present report.

3. Scope

The scope of this comparison was directed towards 3 main objectives:

- to test the competence of the appointed NRLs to analyse complex mixture of phthalates (such as DIDP) in oil as fatty food simulant;

- to validate a preliminary agreed upon analytical method drafted as SOP for determination of DIDP in oil;

- to distinguish between method reproducibility (including sample preparation step) and reproducibility in pure solvent (coming from instrument reproducibility and calibration)

The concentration levels in the oil matrix were chosen in accordance with the legislation [3, 4]

The assessment of the measurement results was undertaken on the basis of requirements laid down in international standards and guidelines [1, 2, 9, 10]

4. Time frame

The EURL asked NRLs to send the description of the method used for determination in oil of DIDP during 2008 ILC so that the EURL could establish an overview and propose the most suitable SOP.

Methods were collected until February 2009. Completed questionnaires were received from 17 laboratories, 7 of which with z-score within the tolerance limits of +/- 2 from 2008, 2 laboratories with strong deviations from the acceptable limits with z-scores > 20 and 4 laboratories which did not present results. A summary of the information obtained from the questionnaires can be found in Annex 1.

The EURL-FCM drafted a proposal for a harmonised SOP based on the most common methods from the best performing laboratories. An improvement was proposed mainly concerning absolute recovery of the DIDP in the acetonitrile extract – by increasing the volume of the acetonitrile for the extraction in order to increase a partitioning coefficient of DIDP between the oil and the acetonitrile.

The draft SOP (Annex 2) was circulated at the beginning of April 2009 to all NRLs for approval. No remarks were received from NRLs which had been established as the communication of a tacit approval.

The oil and acetonitrile samples were prepared at the end of May 2009 following the completion of homogeneity and stability studies by the EURL.

Invitation letters were sent to the laboratories on 25 May 2009 (Annex 3). Laboratories were invited to fill in a letter of confirmation of their participation (Annex 4)

The oil and acetonitrile samples were dispatched to participants on 11 June 2009, together with a letter accompanying the samples (Annex 5), the Standard Operating Procedure of the analytical method to be used for the exercise (Annex 2), detailed instructions for compilation of the results in electronic format (Annex 7), a format for the compilation of results to be eventually sent in non-electronic format (Annex 8) and electronic files where the result should be inserted. The participants were also asked to fill in a letter of confirmation of the receipt of the samples (Annex 6).

Internal Standard solutions were dispatched to the participants on 9 July 2009 due to delay from the supplier in providing the full number of lots of the chemical. Consequently the previously scheduled reporting deadline (31 July 2009) was extended to 12 October. The ILC was closed at end of October after exchange of additional information between the participant and organisers on the correction factor used for re-calculation of the results for DIDP in acetonitrile in mg/kg and on the repeatability/reproducibility conditions for the four replicates.

5. Test material

An industrial source of sunflower oil was used and spiked with DIDP at different levels of concentration by the EURL-FCM. In addition, 3 samples of acetonitrile spiked at different levels of DIDP were prepared by the EURL-FCM

Exercise	Sample	Source
ILC01 2009	**Oil 1 + spike**	Italian oil producer + spike with DIDP (see annex 10)
	Oil 2+ spike	Italian oil producer + spike with DIDP (see annex 10)
	Oil 3 + spike	Italian oil producer + spike with DIDP (see annex 10)
	ACN1 + spike	ACN + spike with DIDP (see annex 10)
	ACN2 + spike	ACN + spike with DIDP (see annex 10)
	ACN3 + spike	ACN + spike with DIDP (see annex 10)

Table 1 . Samples distributed to the participants

Oil:

- 1 bottle of blank oil (50 ml);
- 1 bottle of spiked oil level 1 (50 ml);
- 1 bottle of spiked oil level 2 (50 ml);
- 1 bottle of spiked oil level 3 (50 ml);

Acetonitrile:

- 1 vial of spiked ACN level 1 (10 ml);
- 1 vial of spiked ACN level 2 (10 ml);
- 1 vial of spiked ACN level 3 (10 ml);

In addition to the samples, each participant received two vials with 2 ml of solutions of bis (2-etylhexyl) phthalate-D4 (DEHP-D4) in hexane and in acetonitrile in concentration of 1 mg/kg prepared in the EURL-FCM, to be used as Internal Standard in the collaborative study.

5.1 Preparation

The sunflower oil was purchased from an Italian oil producer and checked for purity.

Preparation and homogenisation of the test material was done by the EURL-FCM laboratory according to the procedure described in Annex 10.

After spiking and homogenisation the oil was dispatched in glass bottles of approximately 100 ml capacity and acetonitrile in screw cap glass vials of 12 ml capacity.

5.2 Homogeneity assessment

The samples were tested for homogeneity by the EURL Laboratory.

Ten randomly selected test specimens for each sample (ACN 1, ACN 2, ACN 3, oil 1, oil 2 and oil 3) were analysed in duplicate for DIDP.

Homogeneity was evaluated by the Prolab Software according to IUPAC International Harmonized Protocol [10] and to the method proposed in the ISO 13528 [1]. The results together with their statistical evaluation are given in Annex 11.

All test materials showed sufficient homogeneity for all the measurands for the target pre-defined standard deviation of the ILC.

5.3 Stability test

Randomly selected specimens for each sample (ACN1, ACN2, ACN3, oil1, oil2 and oil3) were stored at 3 different temperature conditions (+4ºC, room temperature, +40ºC). The test samples were monitored for stability by the EURL, by means of DIDP determination, from 15 May until 5 October 2009. The samples were analysed in duplicate every 3-6 weeks over the given time frame.

Stability was evaluated as described in ISO GUIDE 35:2006 [15].

The evaluation of data was carried out by performing a linear regression on the determined concentrations of DIDP (mean values) vs. time. For a stable material it is expected that the intercept is (within uncertainty) would be equal to the assigned value, whereas the slope does not differ significantly from zero.

Using the linear regression equation:

$$Y_{(\text{DIDP conc, mg/kg})} = b_0 + b_1 X_{(\text{time, weeks})}$$

the slope is not significantly different from zero if the following requirement is respected;

$$|b_1| < t_{0,95,n-2} \cdot s(b_1)$$

Where b1 is the slope obtained from the linear regression, $t_{0.95,n-2}$ is the Student's t-factor for n-2 degrees of freedom and p = 0.95 (95% level of confidence) and $s(b_1)$ is the uncertainty associated with the slope. This can be calculated as fallows:

$$s(b_1) = \frac{s}{\sqrt{\sum_{i=1}^{n}\left(X_i - \bar{X}\right)^2}}$$

The value of s (standard deviation of the points) can be obtained from:

$$s^2 = \frac{\sum_{i=1}^{n} \left(Y_i - b_0 - b_1 X_i\right)^2}{n-2}$$

where n is the number of points of the linear regression.

The results together with their statistical evaluation are given in Annex 12. All test materials showed no significant trend to degradation over the time frame for the ILC01 and for the tested conditions.

5.4 Distribution

The samples were dispatched to the participants by the EURL-FCM on 13 June 2009. Each participant received:

a) A box containing the test materials;

b) An accompanying letter with instructions on sample handling (Annex 5)

c) Instructions to the participant for reporting (Annex 6);

d) A form that had to be sent back after receipt of the sample to confirm its arrival (cf. Annex 7) and

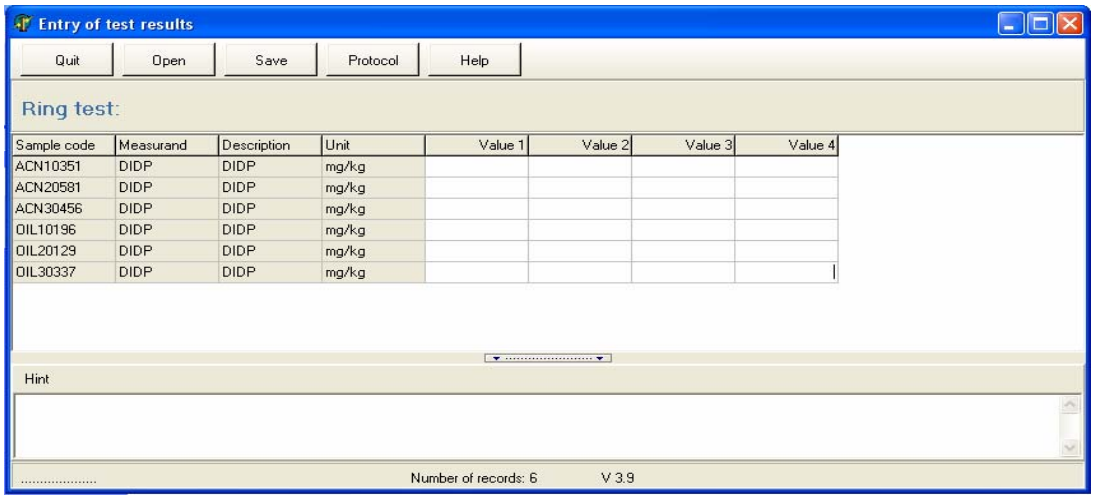
e) A form for reporting the result in non-electronic format (Annex 8)

6. Instructions to participants

Practical instructions were given to all participants in a letter that accompanied the samples (Annex 5).

For the precision experiment the laboratories were asked to perform four replicate measurements and report them. Participants were asked to follow the distributed SOP as close as possible and to report any eventual deviation. The results had to be reported using the measurement units indicated in the instruction letter.

The results were to be reported in a special ProLab [5] software form as shown below:



7. Approaches for statistical evaluation of results

7.1. Evaluation of the DIDP method performance characteristics – methods for determination of the consensus value and repeatability (r) and reproducibility (R) standard deviation

The statistical evaluation of the results was performed using the ProLab software [5] applying different algorithms for the determination of the consensus value and repeatability (r) and reproducibility (R) standard deviation.

The standard ISO 5725-2 [11] is an approach for the statistical analysis of method validation interlaboratory studies, i.e. it should not be used for the analysis of proficiency testing schemes. For the calculations according to those standards the following specific assumptions are made:

- all laboratories (apart from only a very few outlier labs) must have equal analytical performance in order to guarantee that distribution of the test results is close to the normal distribution (whereas this assumption cannot be made for PT's);

- all laboratories must use the same analytical method;

- the method requires replicates.

The standard ISO 5725-2 [11] applies the Grubbs test for the outlier identification of individual test results and laboratory mean values. Additionally tests for the identification of exceeding intra laboratory standard deviations are applied (Cochran test and F-test, respectively). It is a common experience when analysing data from precision experiments to find data that are on the borderline between stragglers and outliers, so that the judgments may have to be made that affect the results of the calculation. This may be unsatisfactory. Applying robust methods as it is described in ISO 5725-5 [14] allows the data to be analysed in such a way that it is not required to make decision that affect the results of the calculations. The algorithm of

ISO 5725-5 (Algorithm A +S) [14] is similar to the one in ISO 13528 [1]. In case those conditions assumed normally for method validation ILC hold and the results are normally distributed, classical statistics in ISO 5725-2 give results that are very similar to robust statistics in 5725-5 or Q/Hampel algorithm in DIN 38402 A45 [7] and ISO/TS 20612 [8].

7.2. Identification of modes using kernel density plotting

Kernel density (KD) plots were additionally used to identify multi-modality in the reported values' distributions.

Frequently analytical results from a collaborative study are not normally distributed or contain values from different populations giving rise to multiple distribution modes. These modes can be visualised by using Kernel density plots [12, 13]. In case the results are not normally distributed the classical statistics from ISO 5725-2 should not be applied

Kernel density plots were computed by the ProLab software [5] from the analytical results by representing the individual numeric values each as a normalised Gaussian distribution centred on the respective analytical value. The sum of these normal distributions forms then the Kernel density distribution. There is a proposal for using a KDM mode as an estimation of the assigned value of one ILC.

7.3. Mandel's h- and k-statistics

Mandel's h-statistic and Mandel's k-statistic [11] present measures for graphically surveying the consistency of the data. They are helpful for method and laboratory assessment. For answering the questions if there are differences between the mean values of the laboratories, Mandel's h-statistic can be considered. In order to assess the variance of each laboratory compared to the variances of the other laboratories, Mandel's k-statistic is useful. Mandel's h- and k- values were calculated by ProLab software following ISO 5725.

The examination of the plots of Mandel's h- and k-statistics may indicate that specific laboratories exhibit patterns of results that are markedly different from the others. This is indicated by (compared to the other laboratories) consistently high or low variation and/or extreme (high or low) mean values.

Various patterns can appear in the plot of Mandel's h-statistic. All laboratories can have both positive and negative values. Individual laboratories may tend to give either all positive or all negative values. This is no unusual pattern, but it may suggest that a common source of laboratory bias exists.

If one laboratory stands out on the k-statistic as having many large values, the respective laboratory has a poorer repeatability precision than the other laboratories. A laboratory could give rise to consistently small k-values because of such factors as excessive rounding of its data or an insensitive measurement scale.

7.4. Evaluation criteria for laboratory performance – type of z-scores

Individual laboratory performance was expressed in terms of z and z'-scores in accordance with ISO 13528 [1] and the International Harmonised Protocol [10]

$$z = \frac{(x_{lab} - X_{assigned})}{\sigma_p}$$

$$z' = \frac{(x_{lab} - X)}{\sqrt{\sigma_p{}^2 + u_{assigned}^2}}$$

where

x_{lab}	is the measurement result reported by a participant
$X_{assigned}$	is the assigned value
σ_p	is the target standard deviation for proficiency assessment
$u_{assigned}$	is the standard uncertainty of the assigned value

The z- and z'-scores can be interpreted as follow:

$\|z\| \leq 2$	satisfactory result
$2 < \|z\| \leq 3$	questionable result
$\|z\| > 3$	unsatisfactory result

The z-scores compared the participant's deviation from the assigned value with the target standard deviation accepted for the ILC σ_p

z'-scores could be used when the assigned value was not calculated using the results reported by the participants. z'-score takes in consideration the uncertainty of the assigned values. In case the guidelines for limiting the uncertainty of the assigned value $u_{assigned} < 0.3\ \sigma_p$ [1] are met, then z'-scores will be similar to z'-scores

When the guideline was not met, the difference in magnitude of the *z'*-scores and *z*-scores may be such that some *z*-scores exceed the critical values of 2,0 or 3,0 and so give "warning signals" by an "action signals", whereas the corresponding *z'*-scores do not exceed these critical values and so do not give signals.

For results reported as "smaller than" (<-values), the reported value was not used in any calculations and no evaluation of the measurement results was made. No scores were given.

7.5. Youden Plot

Youden plots are a graphical technique for analysing ILC data when each laboratory has run test samples in duplicate. It is a simple but effective method for comparing both the within-laboratory variability and the between-laboratory variability.

8. Comments on results and conclusions

8.1 General observations

There were 27 participants from 25 countries to whom samples were dispatched. They all received the samples. The ILC was closed permanently at the end of October for statistical interpretation.

Twenty-four laboratories submitted results. From the EURL-NRL network 22 laboratories out of 24 reported results. There were 2 guests from Germany that provided results as well. As requested, most of the laboratories reported four replicate results under repeatability conditions.

In table 2, a summary of number of participants and test results are shown

Sample	Measurand	Number of test results	Number of laboratories
DIDPACN1	DIDP	90	24
DIDPACN2	DIDP	89	24
DIDPACN3	DIDP	89	24
DIDPOIL1	DIDP	92	25
DIDPOIL2	DIDP	92	25
DIDPOIL3	DIDP	92	25

Table 2: Summary of results of participants and test results.

The participation of the laboratories was regarded as satisfactory for the aim of the precision experiment with regards of the numbers of received results.

8.2 Method performance characteristics from the precision experiment

Precision experiment in one ILC study for method validation has to be conducted with n-replicates analyses in repeatability conditions. After receiving the results from the participants the organisers communicated again with the laboratories with respect to specifications about the conditions in which they performed the 4 replicate analyses, since the instruction letter accompanying the samples had not explicitly requested this information. In order to ensure maximum replies, three successive reminders were sent. The replies were as follows: 16 out of 24 participants performed the replicate analysis under repeatability conditions while 6 participants performed it under reproducibility conditions. 2 participants did not provide any response.

Another issue was the way of expressing results for DIDP content in the solvent (acetonitrile, ACN). Each participant had been contacted directly with the request to express its results in mg/kg without assuming that the density of acetonitrile was 1, as acetonitrile is not a food simulant, but taking into consideration the real density of the solvent at the required temperature.

The results received from the participants were treated statistically twice - once as a

whole batch with all the participants' results and secondly as required for estimation of precision data for the tested method only for the sub-batch of results with replicates obtained only under the repeatability conditions.

Summary of the mean values, reproducibility and repeatability standard deviations (SD) for the first and second batch of test results, calculated according to 3 different algorithms – classical ISO 5725-2, robust ISO 5725-5 and Hampel algorithm (ISO 20612:2007 and DIN 38402 A45) by ProLab software - are given in Table 3.

Figure 1 represents the correlation between the concentration levels and the corresponding repeatability and reproducibility SD.

For repeatability (figure 1a) the data were taken only from the sub-groups of 16 participants who performed the replicates under repeatability conditions since data from the whole batch were not representative for estimation of the repeatability SD. Values calculated according to the 3 different algorithms were very close, but there was a significant difference between repeatability SD in solvent (ACN) and in oil. This was expected and easily explained as for the solvent (ACN) the procedure requires only addition of the internal standard and performing calibration of the instrument. For oil extraction and concentration steps were required before the instrumental analyses, which almost doubled the repeatability SD.

For reproducibility SD the whole batch of data could be used. For estimation of reproducibility SD the classical statistics from ISO 5725-2 were not suitable due to the high number of Grubs and Cochran test outliers and stragglers. Especially for the first level in acetonitrile where there were 7 Cochran outliers the reproducibility SD broke down and increased to 35.7 %. The number and the types of the outliers calculated according to ISO 5725-2 are shown in table 4.

	Grubs outliers (mean value)	Cochran outliers (standard deviation)
DIDPACN1		**7**
DIDPACN2	**1**	**1**
DIDPACN3	**1**	**1**
DIDPOIL1		**3**
DIDPOIL2		**2**
DIDPOIL3	**2**	

Table 4. Number of Grubs and Cochran outliers for all the samples

Both robust algorithms gave very similar results for the mean values and their reproducibility SD (table 3 and figure 1b). The values for reproducibility SD were also very close both when calculated based on the whole batch of data (24 participants) and r-sub-group of 16 participants (table 3 and figure 1b).

For reproducibility SD the values in oil were only slightly higher than in acetonitrile. This leads to the conclusions that under reproducibility conditions the calibration is the main source of random variations as each time a new calibration is performed.

ISO 5725-5	All test results (24 labs)				Reference value, mg/kg	Classical Horwitz, %	Repeatability conditions only (16 labs)			
Sample	Assigned value	R, %	Horrat R	r, %			Assigned value	R, %	Horrat R	r, %
DIDPACN1	2.50	21.3	1.6	3.4	2.51	13.4	2.51	18.2	1.4	2.8
DIDPACN2	6.22	13.3	1.1	3.1	6.33	12.1	6.42	13.3	1.1	2.7
DIDPACN3	9.26	12.9	1.1	2.9	9.40	11.4	9.62	8.8	0.8	2.8
DIDPOIL1	3.47	25.5	1.9	8.7	3.29	13.2	3.59	28.5	2.2	7.0
DIDPOIL2	8.47	14.3	1.2	6.4	8.93	11.6	8.77	14.5	1.2	5.4
DIDPOIL3	12.65	10.2	0.9	6.1	13.35	10.9	12.95	10.5	1.0	5.9

ISO 5725-2	All test results (24 labs)				Reference value, mg/kg	Classical Horwitz, %	Repeatability conditions only (16 labs without Grubs and Cochran outliers)			
Sample	Assigned value	R, %	Horrat R	r, %			Assigned value	R, %	Horrat R	r, %
DIDPACN1	2.62	35.7	2.7	2.3	2.51	13.4	2.42	13.7	1.0	2.6
DIDPACN2	6.25	14.9	1.2	3.8	6.33	12.1	6.44	16.1	1.3	2.6
DIDPACN3	9.22	14.3	1.3	3.2	9.40	11.4	9.70	12.3	1.1	3.1
DIDPOIL1	3.44	25.4	1.9	9.5	3.29	13.2	3.44	22.8	1.7	6.6
DIDPOIL2	8.30	16.1	1.4	6.3	8.93	11.6	8.39	9.2	0.8	4.6
DIDPOIL3	12.62	10.8	1.0	7.2	13.35	10.9	12.76	10.4	1.0	6.0

DIN 38402	All test results (24 labs)				Reference value, mg/kg	Classical Horwitz, %	Repeatability conditions only (16 labs without Grubs and Cochran outliers)			
Sample	Assigned value	R, %	Horrat R	r, %			Assigned value	R, %	Horrat R	r, %
DIDPACN1	2.42	22.3	1.7	3.6	2.51	13.4	2.45	18.4	1.4	3.3
DIDPACN2	6.16	14.9	1.2	3.3	6.33	12.1	6.42	13.7	1.1	2.9
DIDPACN3	9.17	14.5	1.3	3.0	9.40	11.4	9.60	9.2	0.8	3.0
DIDPOIL1	3.47	25.8	2.0	9.1	3.29	13.2	3.57	23.3	1.8	5.6
DIDPOIL2	8.39	15.5	1.3	6.2	8.93	11.6	8.63	13.7	1.2	4.7
DIDPOIL3	12.63	10.8	1.0	5.5	13.35	10.9	12.80	10.5	1.0	4.4

Table 3 Mean values and repeatability/reproducibility SD calculated by 3 different algorithm

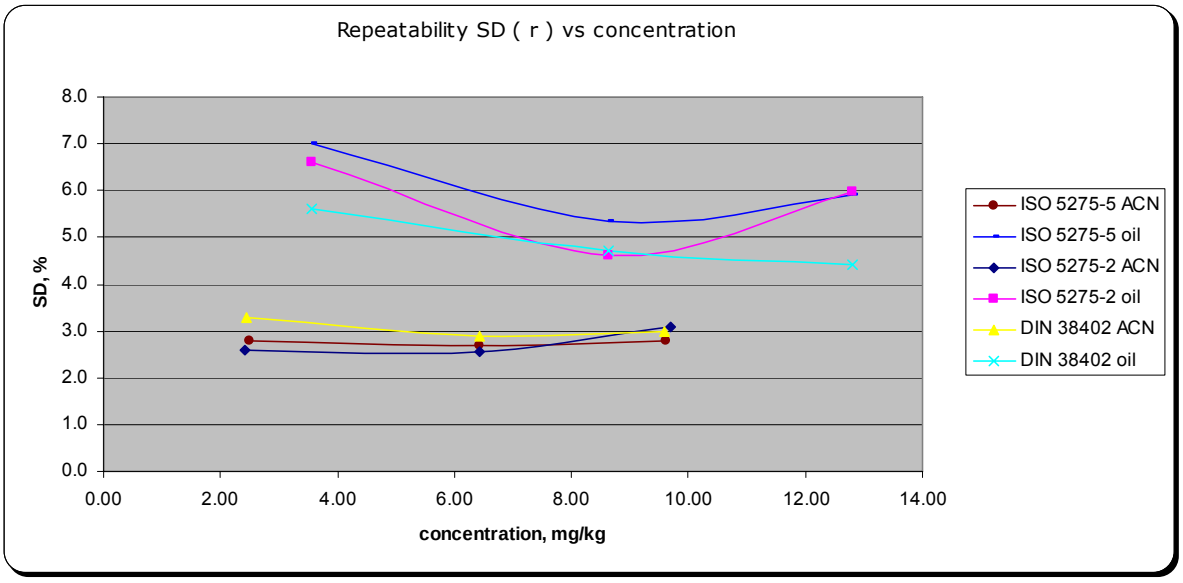


Figure 1a. Repeatability standard deviation versus concentration

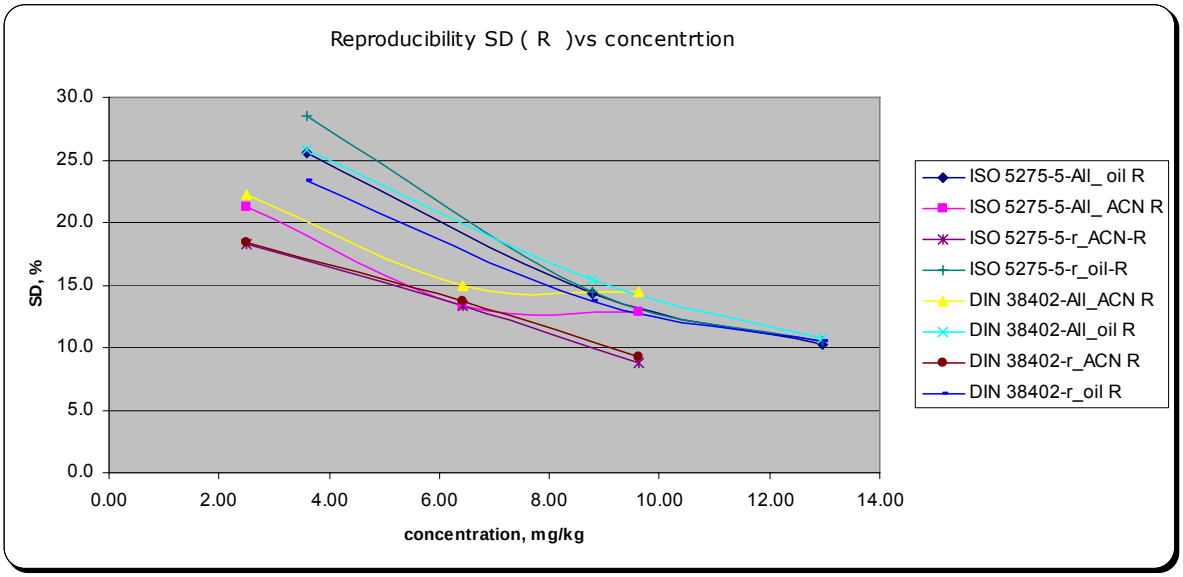


Figure 1b. Reproducibility standard deviation versus concentration

For all samples and concentration levels Horrat R (**HOR**witz**RAT**io) values were calculated according to the formula:

Horrat (R) = Reprod. SD / Predicted Horwitz SD

Horrat value is now one of the acceptability criteria for many of the recently adopted chemical methods of analysis of AOAC INTERNATIONAL, the European Union, and other European organisations dealing with food analysis (e.g., European Committee for Standardisation and Nordic Analytical Committee) [16]. Consistent deviations from the ratio on the low side (values <0.5) may indicate unreported averaging or

excellent training and experience; consistent deviations on the high side (values >2) may indicate inhomogeneity of the test samples, need for further method optimisation or training, operating below the limit of determination, or an unsatisfactory method.

The calculated Horrat values shown in table 3 for all samples and concentration levels correspond to the acceptable limits. It should be pointed out that for the lowest level of DIDP in oil Horrat value is on the upper limit (1.9-2) but this is acceptable as the concentration level is close to the quantification limit of the method.

The robust mean derived from the results coincides well with the reference values calculated based on formulation. The difference between x_{mean} – X_{ref} was less then twice its standard uncertainty for all the levels – table 5.

$$(\frac{(1{,}23s^*)^2}{p} + u_X{}^2)^{1/2}$$, where

u_x is the uncertainty of the reference values;

s* is the robust standard deviation;

p is the number of participating laboratories

ISO 5725-5 (A+S) robust algorithm							
Sample	**Assigned value**	**R, %**	**r, %**	**Reference value**	**difference**	**SD difference**	
DIDPACN1	2.50	21.3	3.35	2.51	-0.01	0.533	TRUE
DIDPACN2	6.22	13.3	3.06	6.33	-0.11	0.828	TRUE
DIDPACN3	9.26	12.9	2.91	9.4	-0.14	1.193	TRUE
DIDPOIL1	3.47	25.5	8.65	3.29	0.18	0.886	TRUE
DIDPOIL2	8.47	14.3	6.39	8.93	-0.46	1.211	TRUE
DIDPOIL3	12.65	10.2	6.12	13.35	-0.70	1.292	TRUE

Table5. Robust mean value against reference values based on formulation

As a result of the precision experiment conducted with ILC01 **DIDP in oil** the following precision parameters could be suggested for the method:

Concentration range	Reproducibility (R), %	Repeatability (r), %
3-6 mg/kg	22 %	6.5 %
6-10 mg/kg	14 %	6 %
> 10 mg/kg	10%	6 %

Table6. Suggested precision parameters for SOP DIDP in oil

8.3 Laboratory results and scores

For calculation of the z-score, hence the performance of each laboratory the most important decision to be taken by the organiser is the assigned value and the target standard deviation against which the performance will be assessed.

For the ***assigned values*** in ILC01 a robust mean was chosen as a consensus assigned value, but the robust mean and the reference values calculated based on formulation were significantly not different.

For the ***target standard deviation*** classical Horwitz SD was assigned for calculation of the Z-scores. As the aim of the present ILC01 was directed more to method validation then to laboratory assessment, z-scores calculated against reproducibility SD as target SD of the ILC, were presented as well.

The results as reported by the participants were summarised in Tables 7 (1-6). Three sets of figures were provided for each of the six samples in Fig 2 (1-6). Each set included (a) individual laboratories values and their mean and standard deviation, (b) the Kernel Density plot, (c) the z- scores.

Figure 2-7 present the results from 2008 for determination of DIDP in oil. As it can be seen for the data on the graphs the harmonisation of the procedure and ***following the same SOP for determination of DIDP in oil in 2009 resulted in this ILC showed a decrease more then 2.5 times in the reproducibility standard deviation from 37% to 14 % for the concentration level around SML of 9 mg/kg while the repeatability SD remained almost the same – 6.5%.***

Additional set of figures of the individual laboratory results and their mean and standard deviation arranged by sub-groups (r- and R-) are presented on Figure 3 (1-6). Red bars are Grubs (B) or Cochran (C) outliers, calculated according ISO 5725-2.

In Fig. 4 Mandel's h- and Mandel's k-statistics are shown as calculated according to ISO 5725-2 for ACN (Fig.4-1) and for oil (Fig 4-2). Values differing statistically significant from values of the other laboratories are marked in a different colour: a red bar indicates a value significant to the significance level of 1% while a yellow bar indicates a value significant to the level of 5%. The outcome of the Mandel's h- and Mandel's k-statistics presented in table 8 are similar to and in correspondence with the Grubs and Cochran outliers' tests according to ISO 5725-2.

Number of laboratories with Mandel h- statistics non consistent with 5% and 1% significance level			
ACN		**OIL**	
1%	5%	1%	5%
0	**4**	**2**	**2**
Number of laboratories with Mandel k- statistics non consistent with 5% and 1% significance level			
ACN		**OIL**	
1%	5%	1%	5%
4	**4**	**2**	**6**

Table 8. Summary of the number of laboratories outliers according to the Mandel tests

Z-scores assessed the laboratory performance against some target standard deviation. As the assigned value was calculated using the results reported by the participants z'-scores could not be used.

Since the present ILC01 was directed mainly towards method validation throughout precision experiment and only then assessment of the laboratory performance, in Figure 5 and Table 9 present z-scores calculated against two different target SD:

a) target SD = classical Horwitz

b) target SD = reproducibility SD of the ILC01

The Youden plot displays a combined graphic of the results of one measurand in two different matrixes. Such a presentation allows identifying systematic effects in the laboratory-specific deviations for both matrixes. It gives an immediate idea of the dominating sources of error in the results. Laboratories having results in the upper left or lower right hand corner of the diagram have analyses dominated by random error. On the other hand, laboratories having results close to the 45° line shown in the plot, but far away from the assigned value have results dominated by systematic error.

Youden plot presented on Figure 6 for the 3 levels of DIDP in acetonitrile against oil shows no correlation between the results because the correlation coefficient is less then 0.5-0.6.

Figure 7 represents the laboratory mean values against its repeatability SD for all concentration levels in acetonitrile and oil. Tolerance limits shown on the graphs were calculated based on classical Horwitz SD. The figure illustrates a clear picture of the results outside the tolerance limits.

Figure 8 represent the overall z-score distribution for all the 147 measurand-matrix-laboratory combination for DIDP in 6 samples and 24 laboratories'. Figure 9 represent them in histogram, like Kernel density plot and normal distribution plot - showing its real normal distribution.

As a conclusion for the laboratory performance, this ILC showed:

- A **noted increase in participation** compared to the similar exercise of 2008. The number of laboratories submitting results for DIDP in oil rose from 17 to 25. This may be due in part to the provision by the EURL of both the method description in a CEN like format as well as of the internal standard.
- A **great increase in laboratory performance** compared to 2008 with 76-92% of successful achievement of results from the participants within the tolerance limits (range 76-92% depended on concentration level considered) compared to 59% in 2008. In particular the performance at the level of the SML was 80% compared to 59% for the same exercise in 2008.

Table 7.1: Laboratories' raw test results, their mean values and corresponding z-score.

Sample:	**1 level DIDP in ACN**	**Rel. target s.d.:**	**14.00% (Horwitz function)**
No. of laboratories:	**24**	**Rel. reproducibility s.d.:**	**22.26%**
Assigned value:	**2.422 mg/kg (Empirical value)**	**Rel. repeatability s.d.:**	**3.60%**

Laboratory	M	M 1	M 2	M 3	M 4	S.d.	Z score
LC0003	1.94	2.03	1.58	2.20	1.94	0.26	-1.43
LC0004	5.50	5.50	5.50	5.51	5.50	0.01	9.09
LC0005	2.14	2.18	2.16	2.13	2.07	0.05	-0.84
LC0006	2.33	2.43	1.91	2.29	2.67	0.32	-0.29
LC0010							
LC0011	5.48	5.48					9.02
LC0012	2.15	2.40	2.10	2.10	2.00	0.17	-0.80
LC0013	2.47	2.45	2.47	2.50	2.44	0.03	0.13
LC0016							
LC0017	2.06	2.05	2.09	2.04	2.06	0.02	-1.07
LC0018	2.03	1.97	1.97	2.07	2.09	0.06	-1.17
LC0020	3.75	3.63	4.09	3.78	3.50	0.25	3.92
LC0021	2.45	2.40	2.40	2.60	2.40	0.10	0.08
LC0025	2.67	2.62	2.72	2.65	2.71	0.05	0.75
LC0028	2.60	2.60	2.70	2.50	2.60	0.08	0.53
LC0029							
LC0031	1.63	1.70	1.60	1.60	1.60	0.05	-2.35
LC0037	2.27	2.17	2.90	1.74	2.27	0.48	-0.45
LC0038	2.42	2.46	2.39	2.42	2.42	0.03	0.00
LC0040	2.90	2.90	2.90	2.90	2.90	0.00	1.41
LC0041	2.58	2.50	2.60	2.50	2.70	0.10	0.45
LC0043							
LC0044							
LC0048	1.91	1.91					-1.51
LC0049	2.38	2.35	2.38	2.38	2.41	0.02	-0.12
LC0050	3.35	3.60	3.70	3.60	2.50	0.57	2.74
LC0052							
LC0054	2.56	2.47	2.54	2.56	2.65	0.07	0.39
LC0055	2.42	2.45	2.53	2.37	2.32	0.09	-0.01
LC0056	2.94	3.27	3.25	2.61	2.62	0.37	1.52

Table 7.2: Laboratories' raw test results, their mean values and corresponding z-score.

Sample:	**2 level DIDP in ACN**	**Rel. target s.d.:**	**12.17% (Horwitz function)**
No. of laboratories:	**24**	**Rel. reproducibility s.d.:**	**14.94%**
Assigned value:	**6.164 mg/kg (Empirical value)**	**Rel. repeatability s.d.:**	**3.28%**

Laboratory	M	M 1	M 2	M 3	M 4	S.d.	Z score
LC0003	6.88	6.51	7.14	6.42	7.46	0.50	0.96
LC0004	8.54	8.42	8.65	8.44	8.66	0.13	3.17
LC0005	5.88	5.86	6.03	5.87	5.75	0.11	-0.38
LC0006	5.89	5.89	6.54	5.36	5.78	0.49	-0.36
LC0010							
LC0011	14.60	14.60					11.25
LC0012	5.68	5.70	6.10	5.50	5.40	0.31	-0.65
LC0013	6.04	6.05	5.99	5.97	6.13	0.07	-0.17
LC0016							
LC0017	4.90	5.03	4.92	4.86	4.78	0.10	-1.69
LC0018	5.47	5.53	5.55	5.41	5.41	0.08	-0.92
LC0020	8.13	8.36	8.34	7.82	7.98	0.27	2.61
LC0021	6.83		6.80	6.80	6.90	0.06	0.89
LC0025	6.20	6.11	6.50	6.25	5.92	0.24	0.04
LC0028	7.18	7.10	7.40	7.20	7.00	0.17	1.35
LC0029							
LC0031	4.75	4.60	4.70	4.70	5.00	0.17	-1.89
LC0037	5.40	6.41	5.19	4.61	5.40	0.75	-1.02
LC0038	6.13	6.20	6.12	6.17	6.03	0.07	-0.05
LC0040	6.40	6.40	6.40	6.40	6.40	0.00	0.31
LC0041	6.28	6.40	6.60	6.00	6.10	0.28	0.15
LC0043							
LC0044							
LC0048	6.63	6.63					0.62
LC0049	5.64	5.55	5.78	5.57	5.64	0.10	-0.71
LC0050	5.93	6.20	5.90	6.00	5.60	0.25	-0.32
LC0052							
LC0054	6.00	6.04	5.98	6.03	5.93	0.05	-0.23
LC0055	5.92	6.00	5.84	6.09	5.74	0.16	-0.33
LC0056	6.65	6.74	6.26	6.50	7.11	0.36	0.65

Table 7.3: Laboratories' raw test results, their mean values and corresponding z-score

Sample:	**3 level DIDP in ACN**	**Rel. target s.d.:**	**11.46% (Horwitz function)**
No. of laboratories:	**24**	**Rel. reproducibility s.d.:**	**14.49%**
Assigned value:	**9.170 mg/kg (Empirical value)**	**Rel. repeatability s.d.:**	**3.04%**

Laboratory	M	M 1	M 2	M 3	M 4	S.d.	Z score
LC0003	10.09	10.60	10.10	9.25	10.40	0.59	0.87
LC0004	10.43	10.32	10.53	10.32	10.55	0.13	1.20
LC0005	9.14	9.06	9.16	9.07	9.28	0.10	-0.03
LC0006	9.44	9.03	9.95	9.21	9.55	0.41	0.25
LC0010							
LC0011	22.02	22.02					12.23
LC0012	8.48	8.60	8.60	8.40	8.30	0.15	-0.66
LC0013	9.11	8.97	9.19	9.14	9.15	0.10	-0.05
LC0016							
LC0017	7.09	7.51	7.14	7.16	6.55	0.40	-1.98
LC0018	6.83	7.15	7.16	6.50	6.50	0.38	-2.23
LC0020	11.32	11.02	11.70	11.26	11.30	0.28	2.05
LC0021	10.03		10.20	10.10	9.80	0.21	0.82
LC0025	9.34	9.10	9.53	9.39	9.36	0.18	0.17
LC0028	12.50	12.40	12.10	12.70	12.80	0.32	3.17
LC0029							
LC0031	7.60	7.80	7.50	7.60	7.50	0.14	-1.49
LC0037	8.39	9.80	7.50	7.85	8.39	1.01	-0.75
LC0038	9.65	9.91	9.10	9.64	9.96	0.39	0.46
LC0040	9.25	9.00	9.00	9.50	9.50	0.29	0.08
LC0041	9.18	9.10	9.00	9.60	9.00	0.29	0.00
LC0043							
LC0044							
LC0048	9.95	9.95					0.74
LC0049	9.08	9.04	8.96	9.13	9.20	0.10	-0.08
LC0050	8.05	8.10	7.90	8.20	8.00	0.13	-1.07
LC0052							
LC0054	8.78	8.76	8.90	8.90	8.56	0.16	-0.37
LC0055	9.28	9.94	9.27	8.76	9.14	0.49	0.10
LC0056	8.97	8.90	9.07	8.87	9.02	0.10	-0.19

Table 7.4: Laboratories' raw test results, their mean values and corresponding z-score

Sample:	**1 level DIDP in oil**	**Rel. target s.d.:**	**13.26% (Horwitz function)**
No. of laboratories:	**25**	**Rel. reproducibility s.d.:**	**25.79%**
Assigned value:	**3.475 mg/kg (Empirical value)**	**Rel. repeatability s.d.:**	**9.14%**

Laboratory	M	M 1	M 2	M 3	M 4	S.d.	Z score
LC0003	5.4725	4.9100	5.3500	5.0900	6.5400	0.7342	4.3355
LC0004	2.1550	2.3200	1.9900			0.2333	-2.8635
LC0005	3.4406	3.4430	3.1711	3.8947	3.2535	0.3234	-0.0738
LC0006	3.1625	2.3700	3.3800	3.8100	3.0900	0.6055	-0.6772
LC0010							
LC0011	3.3800	3.2800	3.4800			0.1414	-0.2052
LC0012	3.6250	3.2000	4.0000	3.7000	3.6000	0.3304	0.3264
LC0013	3.4550	3.6700	3.3100	3.4500	3.3900	0.1544	-0.0425
LC0016	4.7750	4.6000	3.1000	6.0000	5.4000	1.2553	2.8219
LC0017	2.8750	3.0000	2.5000	3.4000	2.6000	0.4113	-1.3011
LC0018	2.6175	2.8100	2.8300	2.4300	2.4000	0.2343	-1.8599
LC0020	4.9675	4.9400	4.6600	5.3500	4.9200	0.2851	3.2397
LC0021	2.9250	3.0000	2.7000	2.9000	3.1000	0.1708	-1.1926
LC0025	3.3600	3.4000	3.4800	3.5200	3.0400	0.2191	-0.2486
LC0028	3.8725	4.4400	3.4900	3.8900	3.6700	0.4122	0.8635
LC0029							
LC0031	3.5750	3.2000	3.7000	3.8000	3.6000	0.2630	0.2179
LC0037	3.6100	1.6800	3.4600	4.2300	5.0700	1.4449	0.2939
LC0038	3.2200	3.0800	3.2600	3.1600	3.3800	0.1296	-0.5524
LC0040	2.4000	2.3000	2.4000	2.5000	2.4000	0.0816	-2.3319
LC0041	4.7750	4.5000	4.6000	4.9000	5.1000	0.2754	2.8219
LC0043							
LC0044							
LC0048	4.4000	4.4000					2.0082
LC0049	3.2225	3.1700	3.1700	3.3100	3.2400	0.0670	-0.5470
LC0050	4.0667	3.9000	3.9000	4.4000		0.2887	1.2848
LC0052							
LC0054	2.8250	3.3600	2.8500	2.3200	2.7700	0.4262	-1.4096
LC0055	2.8275	2.9000	2.8700	2.7600	2.7800	0.0680	-1.4042
LC0056	2.6625	2.8800	2.4700	2.6300	2.6700	0.1688	-1.7622

Table 7.5: Laboratories' raw test results, their mean values and corresponding z-score

Sample:	**2 lelev DIDP in oil**	**Rel. target s.d.:**	**11.61% (Horwitz function)**
No. of laboratories:	**25**	**Rel. reproducibility s.d.:**	**15.46%**
Assigned value:	**8.394 mg/kg (Empirical value)**	**Rel. repeatability s.d.:**	**6.22%**

Laboratory	M	M 1	M 2	M 3	M 4	S.d.	Z score
LC0003	7.81	6.76	7.72	8.36	8.41	0.77	-0.60
LC0004	7.05	6.66	7.43			0.54	-1.38
LC0005	7.61	8.06	7.78	7.23	7.37	0.38	-0.80
LC0006	7.34	6.83	7.99	7.41	7.13	0.49	-1.08
LC0010							
LC0011	8.80	8.55	9.05			0.35	0.42
LC0012	8.25	9.50	7.50	7.80	8.20	0.88	-0.15
LC0013	9.25	10.03	9.21	8.64	9.13	0.58	0.88
LC0016	18.45	15.30	19.00	19.40	20.10	2.15	10.31
LC0017	7.70	9.20	5.80	8.10	7.70	1.42	-0.71
LC0018	5.11	5.56	4.21	5.27	5.39	0.61	-3.37
LC0020	9.69	9.66	9.80	9.52	9.76	0.12	1.32
LC0021	7.68	8.10	7.20	7.70	7.70	0.37	-0.74
LC0025	8.86	8.64	8.94	8.85	8.99	0.15	0.47
LC0028	10.46	9.39	10.14	12.78	9.51	1.58	2.11
LC0029							
LC0031	8.55	8.60	8.40	8.80	8.40	0.19	0.16
LC0037	7.54	6.78	7.24	7.73	8.41	0.70	-0.88
LC0038	8.66	8.67	8.94	8.46	8.56	0.21	0.27
LC0040	8.18	7.70	8.40	8.50	8.10	0.36	-0.22
LC0041	10.57	9.10	10.80	11.70	10.70	1.08	2.24
LC0043							
LC0044							
LC0048	8.87	8.87					0.49
LC0049	8.53	8.58	8.45	8.51	8.56	0.06	0.13
LC0050	11.40	11.80	11.00	11.40		0.40	3.08
LC0052							
LC0054	7.64	7.93	8.17	7.47	6.97	0.53	-0.78
LC0055	8.04	8.43	8.30	7.69	7.74	0.38	-0.36
LC0056	7.97	8.47	7.81	7.54	8.06	0.40	-0.44

Table 7.6: Laboratories' raw test results, their mean values and corresponding z-score

Sample:	**3 level DIDP in oil**	**Rel. target s.d.:**	**10.92% (Horwitz function)**
No. of laboratories:	**25**	**Rel. reproducibility s.d.:**	**10.76%**
Assigned value:	**12.635 mg/kg (Empirical value)**	**Rel. repeatability s.d.:**	**5.47%**

Laboratory	M	M 1	M 2	M 3	M 4	S.d.	Z score
LC0003	13.90	12.65	12.92	14.33	15.70	1.41	0.92
LC0004	9.16	8.16	10.16			1.41	-2.52
LC0005	11.33	11.14	11.16	11.19	11.82	0.33	-0.95
LC0006	10.55	11.07	10.15	10.57	10.42	0.39	-1.51
LC0010							
LC0011	15.25	15.13	15.37			0.17	1.90
LC0012	13.90	12.40	16.70	13.00	13.50	1.92	0.92
LC0013	12.76	13.73	12.27	12.24	12.79	0.70	0.09
LC0016	24.00	24.70	26.50	21.30	23.50	2.18	8.24
LC0017	12.45	11.90	12.60	12.80	12.50	0.39	-0.13
LC0018	6.68	6.55	6.25	6.54	7.37	0.48	-4.32
LC0020	12.49	12.57	12.69	13.01	11.69	0.56	-0.10
LC0021	12.48	12.50	12.10	12.60	12.70	0.26	-0.12
LC0025	13.09	12.86	13.11	13.40	12.99	0.23	0.33
LC0028	14.55	13.00	16.08	14.55	14.56	1.26	1.39
LC0029							
LC0031	12.08	12.50	12.00	11.50	12.30	0.43	-0.41
LC0037	13.01	12.02	12.57	15.02	12.41	1.36	0.27
LC0038	12.88	13.01	12.97	13.36	12.18	0.50	0.18
LC0040	13.20	14.30	13.60	12.40	12.50	0.91	0.41
LC0041	13.10	14.40	13.50	12.30	12.20	1.05	0.34
LC0043							
LC0044							
LC0048	12.92	12.92					0.21
LC0049	12.35	12.71	12.76	12.06	11.88	0.45	-0.20
LC0050	12.10	11.50	11.70	13.10		0.87	-0.39
LC0052							
LC0054	12.39	12.00	14.69	11.03	11.85	1.59	-0.18
LC0055	12.26	12.31	12.78	12.01	11.94	0.38	-0.27
LC0056	11.67	11.59	11.99	11.63	11.46	0.23	-0.70

Figure 2-1: Summary graphs of the laboratory's test results with their repeatability SD (a), Kernel Density plot (b) and z-scores (c)

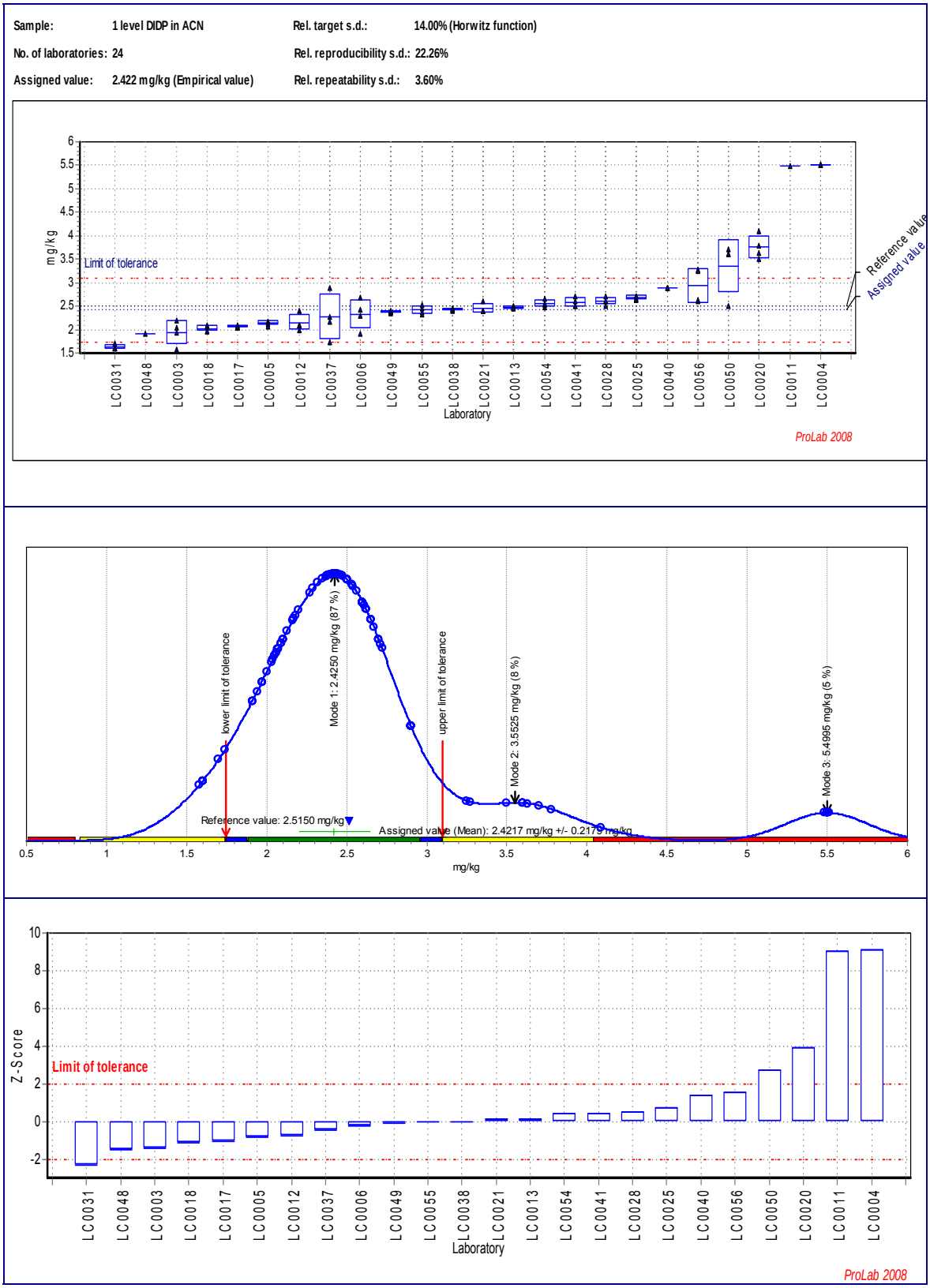


Figure 2-2: Summary graphs of the laboratory's test results with their repeatability SD (a), Kernel Density plot (b) and z-scores (c)

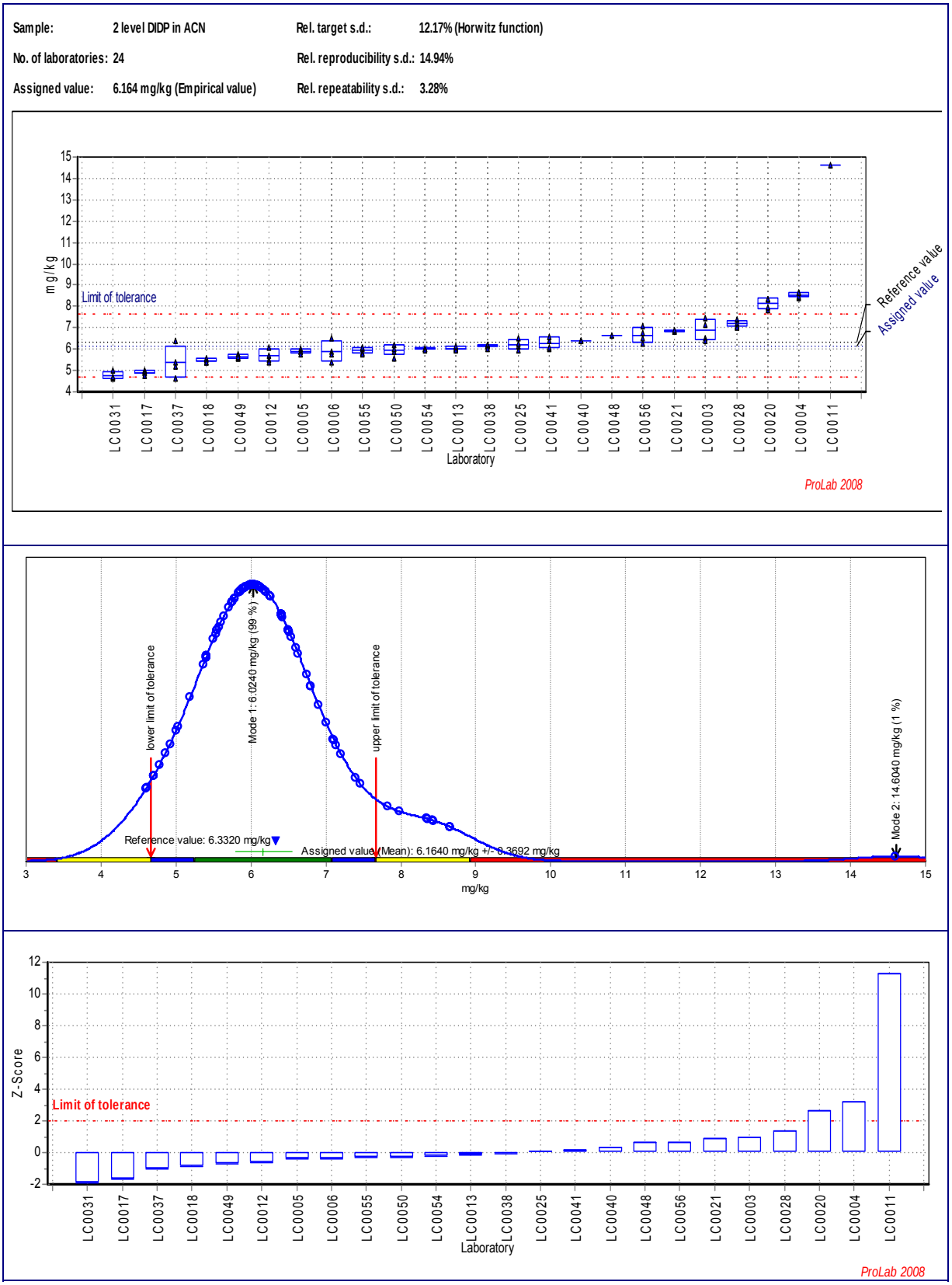


Figure 2-3: Summary graphs of the laboratory's test results with their repeatability SD (a), Kernel Density plot (b) and z-scores (c)

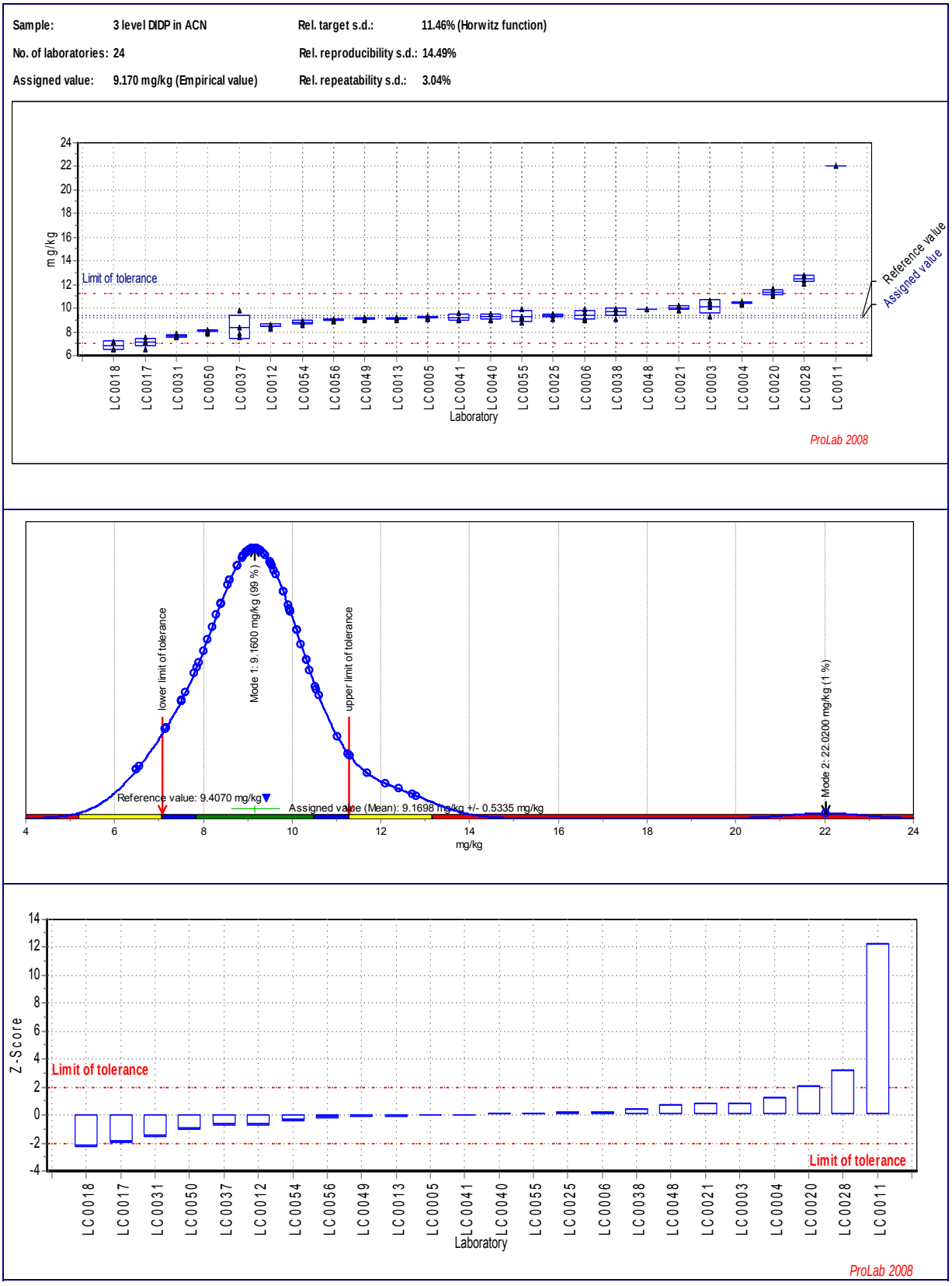


Figure 2-4: Summary graphs of the laboratory's test results with their repeatability SD (a), Kernel Density plot (b) and z-scores (c)

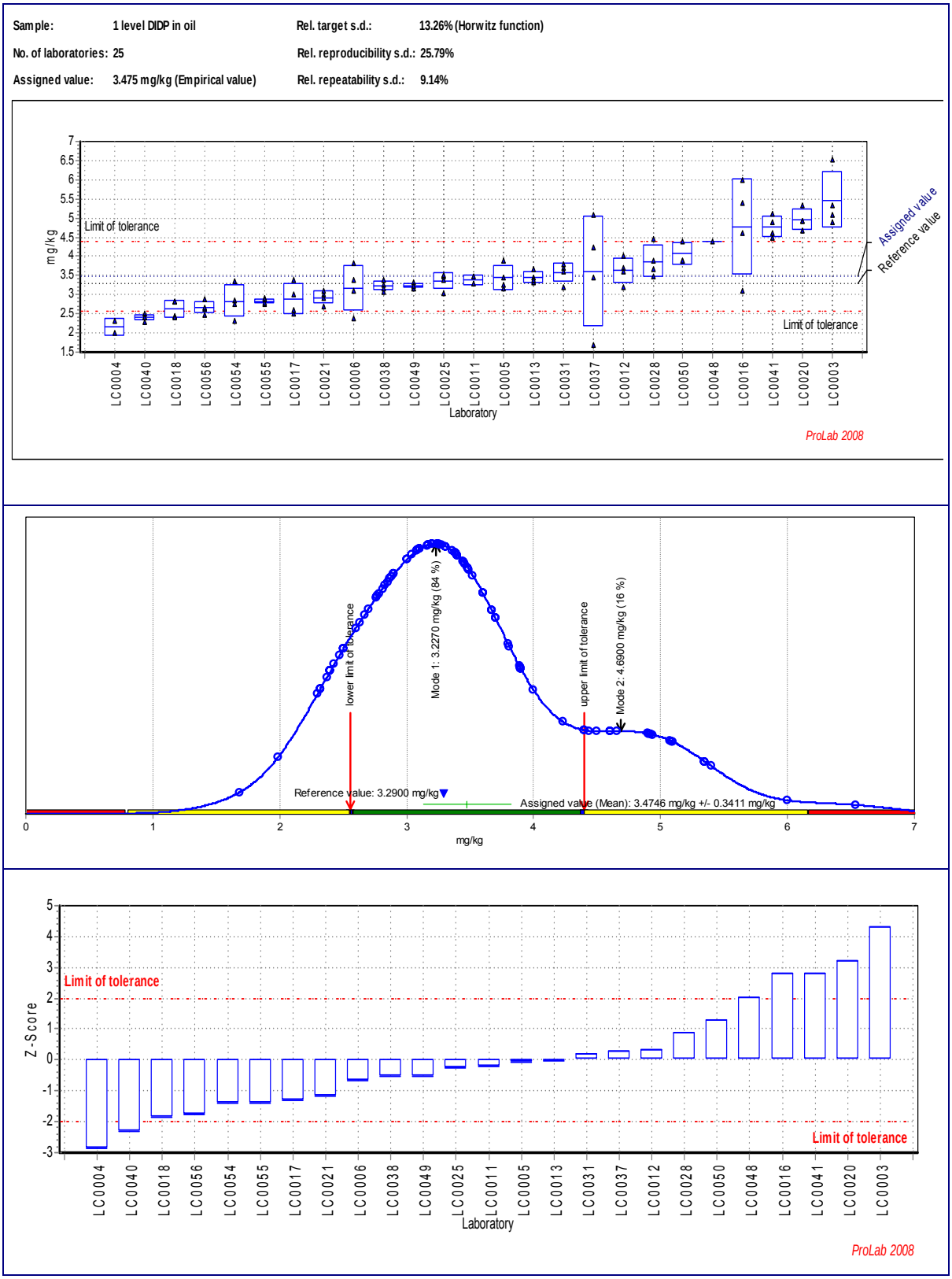


Figure 2-5: Summary graphs of the laboratory's test results with their repeatability SD (a), Kernel Density plot (b) and z-scores (c)

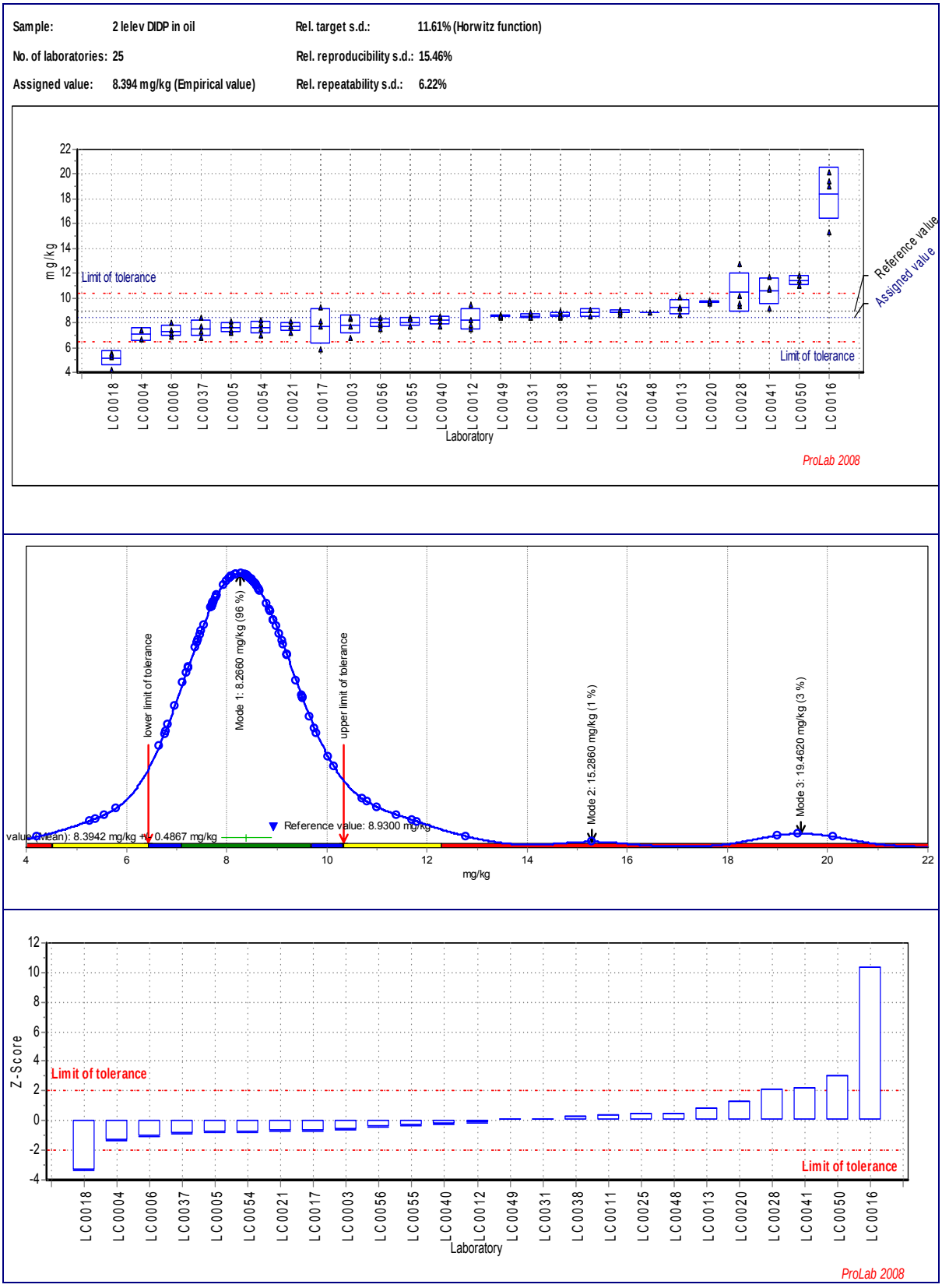


Figure 2-6: Summary graphs of the laboratory's test results with their repeatability SD (a), Kernel Density plot (b) and z-scores (c)

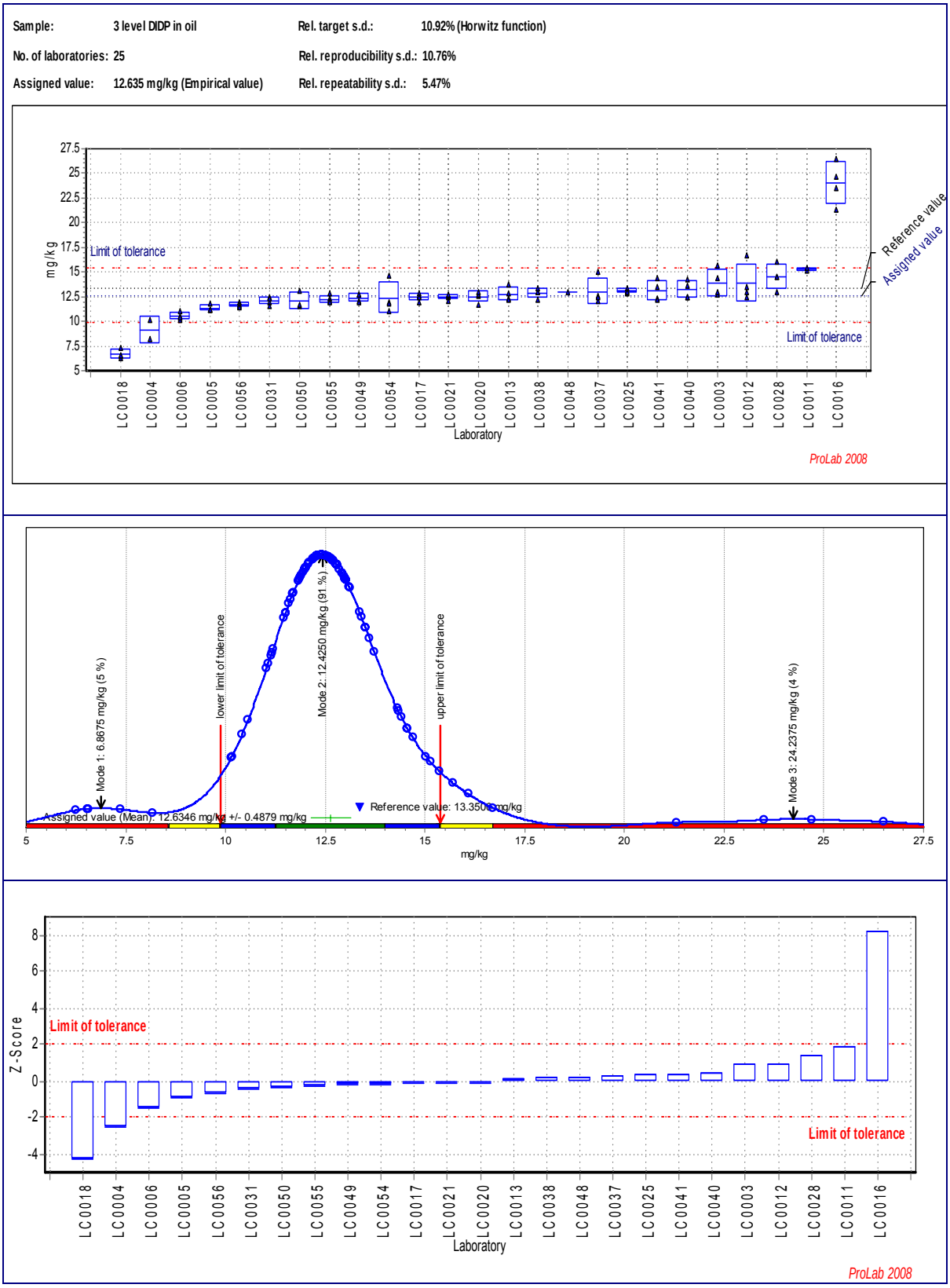


Figure 2-7: Summary graphs of the laboratory's test results with their repeatability SD (a), Kernel Density plot (b) and z'-scores (c) - 2008 r=6.6%

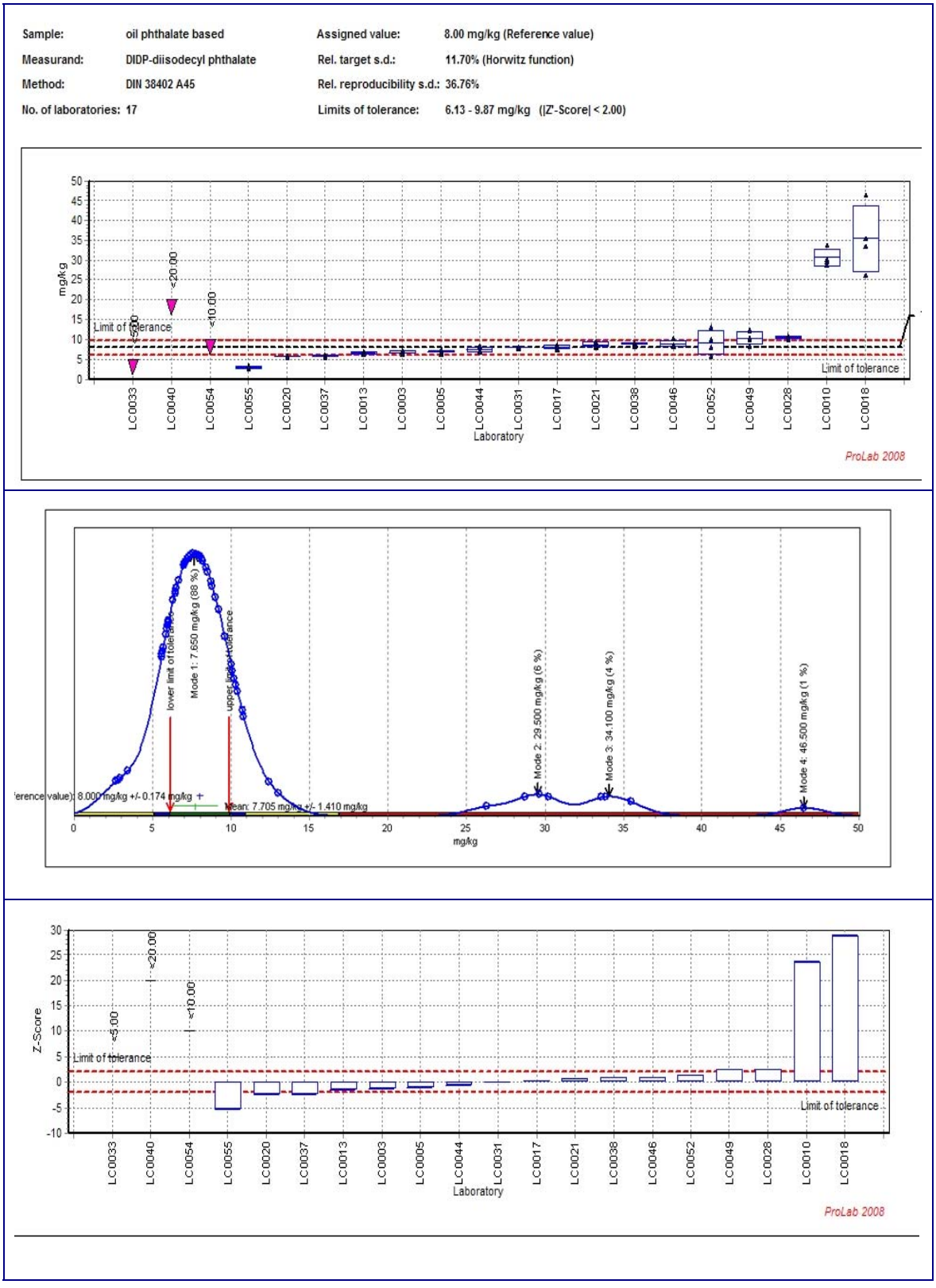


Figure 3 Distribution of mean values and its SD arranged by sub-groups of repeatability/reproducibility conditions of the 4 replicates (some laboratories did notprovide the information)

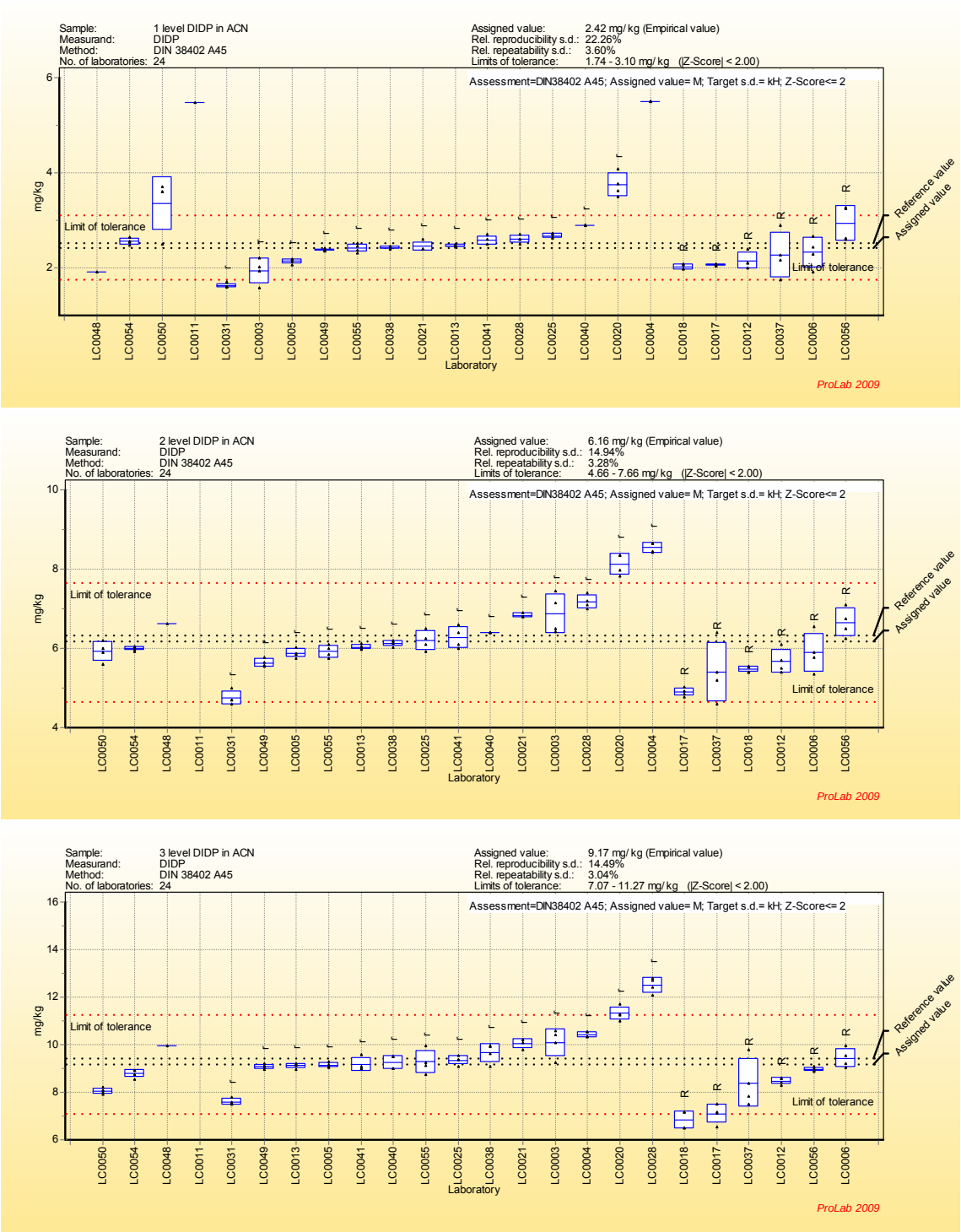
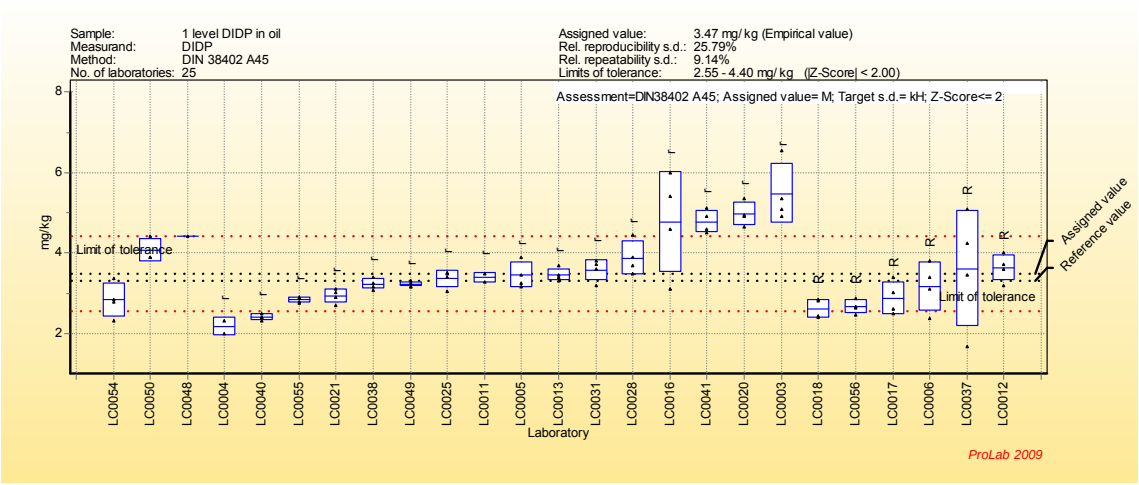
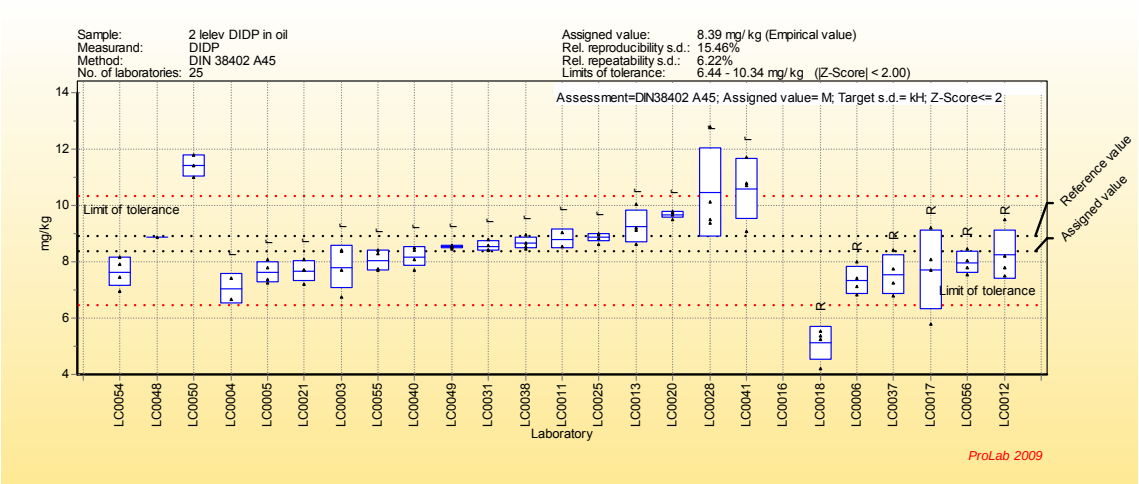


Figure 3 (contnd) Distribution of mean values and its SD arranged by sub-groups of repeatability/reproducibility conditions of the 4 replicates (some laboratories did notprovide the information)





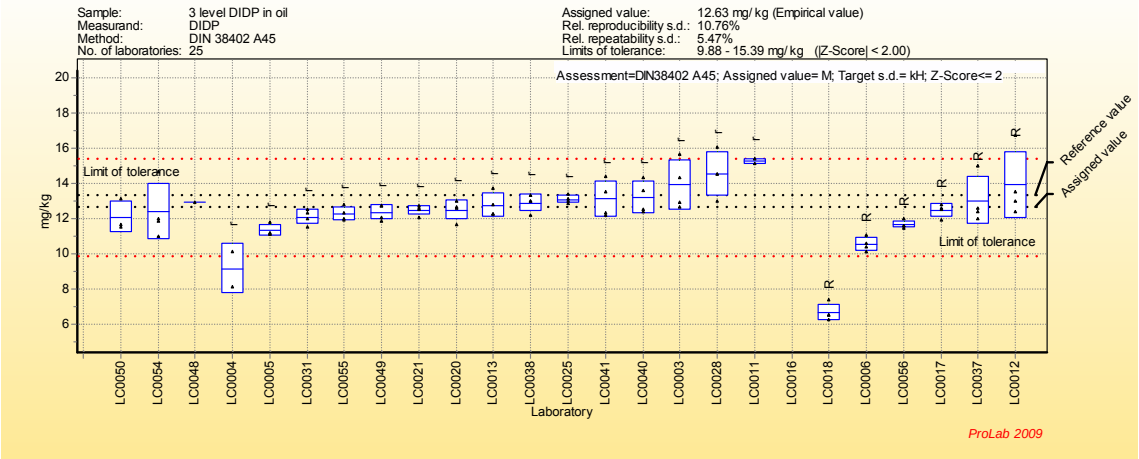
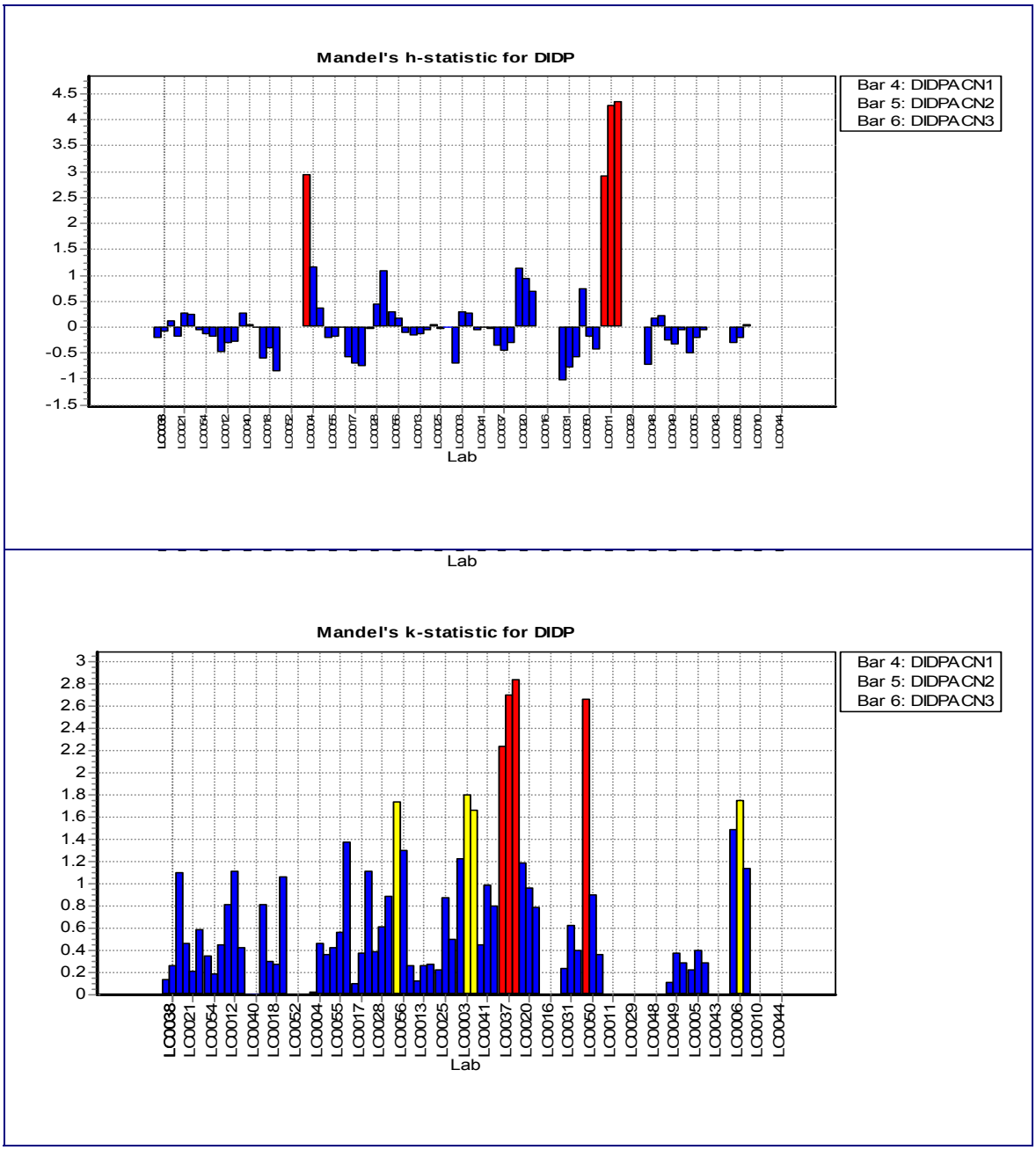
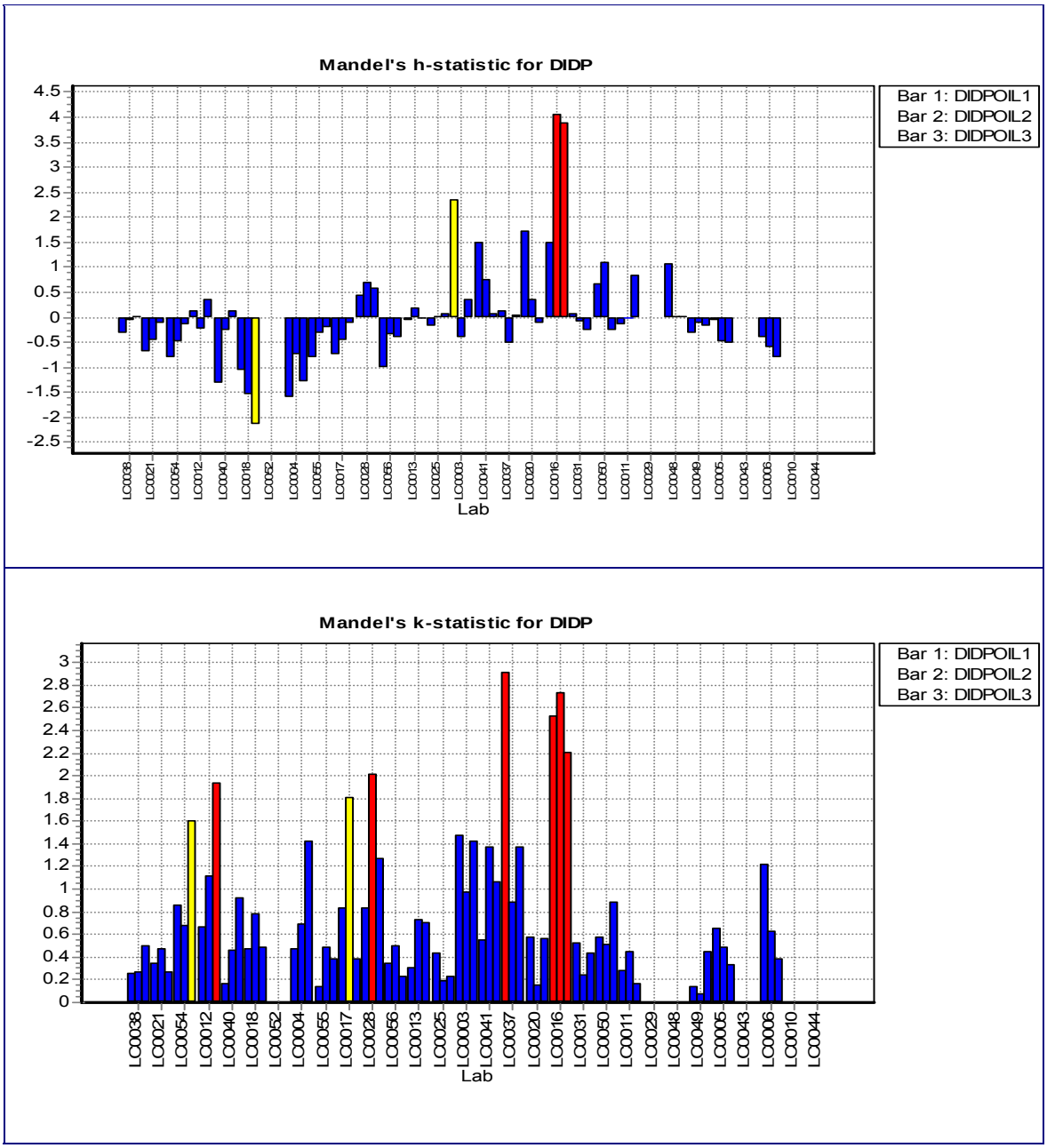


Figure 4-1:. Mandel h- and k-statistics for DIDP in ACN



* for 1% significant level the indicative Mandel's h value is 2.43 and k-value (for n=4 replicates) is 1.90. Laboratories with higher values are marked in red
* for 5% significant level the indicative Mandel's h value is 1.90 and k-value (for n=4 replicates) is 1.60. Laboratories with higher values are marked in yellow
** The legend next to the figure explains the sequence of the bars for each laboratory, i.e. the first entry in the legend coincides with the bar at the farthest-left (for one laboratory), while the last legend entry coincides with the bar on the farthest-right (for one laboratory).

Figure 4-2: Mandel h- and k-statistics for DIDP in oil



* for 1% significant level the indicative Mandel's h value is 2.43 and k-value (for n=4 replicates) is 1.90 Laboratories with higher values are marked in red
* for 5% significant level the indicative Mandel's h value is 1.90 and k-value (for n=4 replicates) is 1.60 Laboratories with higher values are marked in yellow
** The legend next to the figure explains the sequence of the bars for each laboratory, i.e. the first entry in the legend coincides with the bar at the farthest-left (for one laboratory), while the last legend entry coincides with the bar on the farthest-right (for one laboratory).

Table 9: Summary of z -scores against target SD

	Target SD (Classical Horwitz)						Target SD (ILC01 reprod. SD)					
	DIDPACN1	DIDPACN2	DIDPACN3	DIDPOIL1	DIDPOIL2	DIDPOIL3	DIDPACN1	DIDPACN2	DIDPACN3	DIDPOIL1	DIDPOIL2	DIDPOIL3
%	**14**	**12.16**	**11.46**	**13.26**	**11.61**	**10.92**	**22.26**	**14.94**	**14.49**	**25.78**	**15.46**	**10.75**
Laboratory												
LC0003	-1.43	0.96	0.87	**4.34**	-0.6	0.92	-0.9	0.78	0.69	**2.23**	-0.45	0.93
LC0004	**9.09**	**3.17**	1.2	**-2.86**	-1.38	**-2.52**	**5.71**	**2.58**	0.95	-1.47	-1.04	**-2.56**
LC0005	-0.84	-0.38	-0.03	-0.07	-0.8	-0.95	-0.53	-0.31	-0.02	-0.04	-0.6	-0.96
LC0006	-0.29	-0.36	0.25	-0.68	-1.08	-1.51	-0.18	-0.29	0.2	-0.35	-0.81	-1.53
LC0011	**9.02**	**11.25**	**12.23**	-0.21	0.42	1.9	**5.67**	**9.16**	**9.67**	-0.11	0.31	1.92
LC0012	-0.8	-0.65	-0.66	0.33	-0.15	0.92	-0.5	-0.53	-0.52	0.17	-0.11	0.93
LC0013	0.13	-0.17	-0.05	-0.04	0.88	0.09	0.08	-0.14	-0.04	-0.02	0.66	0.09
LC0016				**2.82**	**10.31**	**8.24**				1.45	**7.75**	**8.36**
LC0017	-1.07	-1.69	**-1.98**	-1.3	-0.71	-0.13	-0.67	-1.38	-1.57	-0.67	-0.53	-0.14
LC0018	-1.17	-0.92	**-2.23**	-1.86	**-3.37**	**-4.32**	-0.74	-0.75	-1.76	-0.96	**-2.53**	**-4.38**
LC0020	**3.92**	**2.61**	**2.05**	**3.24**	1.32	-0.1	**2.46**	**2.13**	1.62	1.67	0.99	-0.11
LC0021	0.08	0.89	0.82	-1.19	-0.74	-0.12	0.05	0.73	0.65	-0.61	-0.55	-0.12
LC0025	0.75	0.04	0.17	-0.25	0.47	0.33	0.47	0.03	0.13	-0.13	0.35	0.34
LC0028	0.53	1.35	**3.17**	0.86	**2.11**	1.39	0.33	1.1	**2.51**	0.44	1.59	1.41
LC0031	**-2.35**	-1.89	-1.49	0.22	0.16	-0.41	-1.48	-1.54	-1.18	0.11	0.12	-0.41
LC0037	-0.45	-1.02	-0.75	0.29	-0.88	0.27	-0.28	-0.83	-0.59	0.15	-0.66	0.27
LC0038		-0.05	0.46	-0.55	0.27	0.18		-0.04	0.36	-0.28	0.2	0.18
LC0040	1.41	0.31	0.08	**-2.33**	-0.22	0.41	0.89	0.26	0.06	-1.2	-0.17	0.42
LC0041	0.45	0.15	0	**2.82**	**2.24**	0.34	0.28	0.12	0	1.45	1.68	0.34
LC0048	-1.51	0.62	0.74	**2**	0.49	0.21	-0.95	0.51	0.59	1.03	0.37	0.21
LC0049	-0.12	-0.71	-0.08	-0.55	0.13	-0.2	-0.08	-0.57	-0.07	-0.28	0.1	-0.21
LC0050	**2.74**	-0.32	-1.07	1.28	**3.08**	-0.39	1.72	-0.26	-0.84	0.66	**2.32**	-0.39
LC0054	0.39	-0.23	-0.37	-1.41	-0.78	-0.18	0.25	-0.18	-0.29	-0.72	-0.58	-0.18
LC0055	-0.01	-0.33	0.1	-1.4	-0.36	-0.27	-0.01	-0.27	0.08	-0.72	-0.27	-0.28
LC0056	1.52	0.65	-0.19	-1.76	-0.44	-0.7	0.96	0.53	-0.15	-0.91	-0.33	-0.71

Table 9 (continue): Summary of z-scores against target SD

	Target SD (Classical Horwitz)						Target SD (ILC01 reprod. SD)					
Number of laboratories	DIDPACN1	DIDPACN2	DIDPACN3	DIDPOIL1	DIDPOIL2	DIDPOIL3	DIDPACN1	DIDPACN2	DIDPACN3	DIDPOIL1	DIDPOIL2	DIDPOIL3
with z>2	2	1	3	4	2	1	1	2	1	1	2	1
with z>3	3	2	2	2	3	2	2	1	1	0	1	2
Total N	24	24	24	25	25	25	24	24	24	25	25	25
% succesful	**79.2**	**87.5**	**79.2**	**76.0**	**80.0**	**88.0**	**87.5**	**87.5**	**91.7**	**96.0**	**88.0**	**88.0**

Figure 5: Summary of z –scores target SD = reproducibility SD

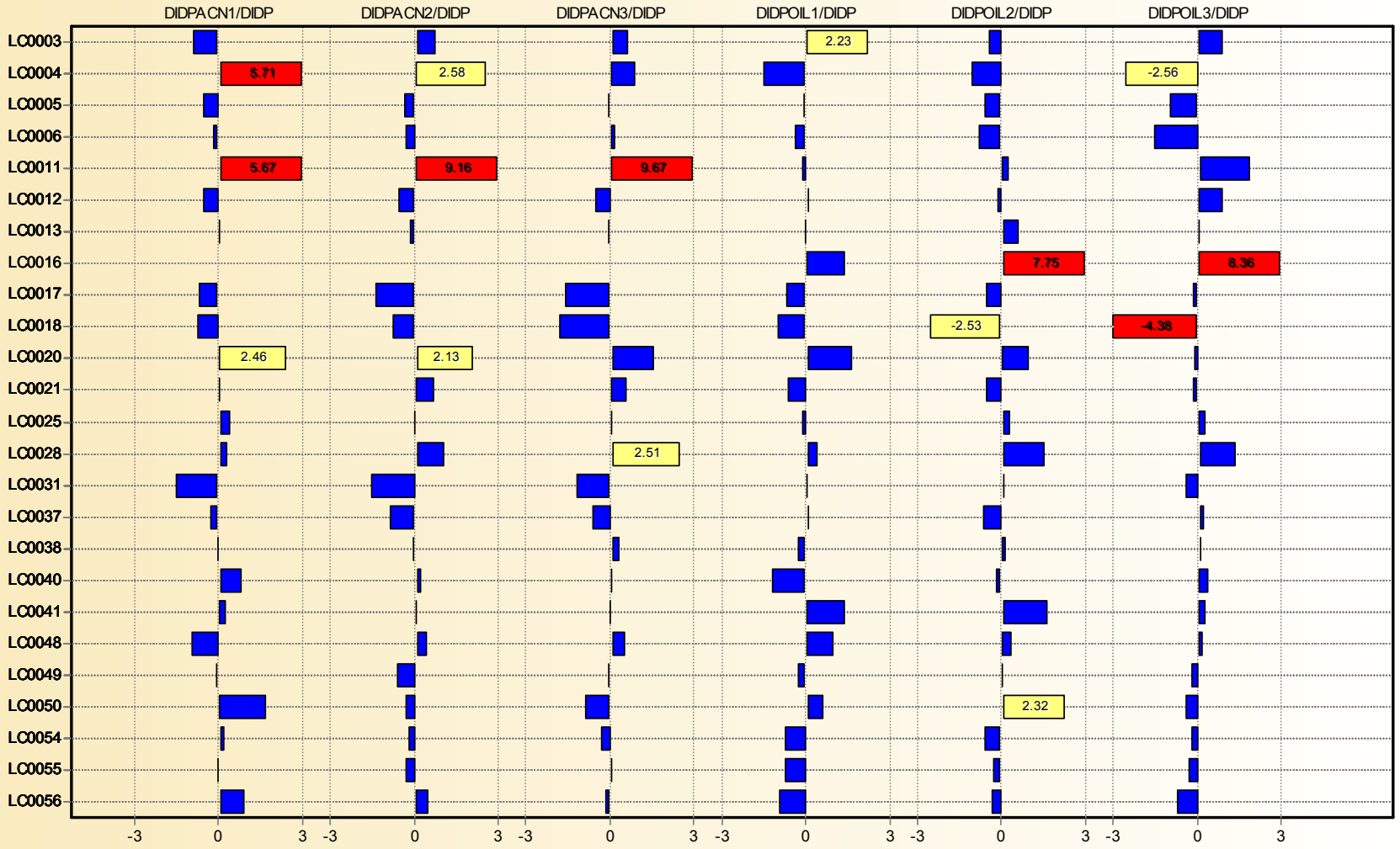


Figure 5: Summary of z – scores against target SD = Horwitz

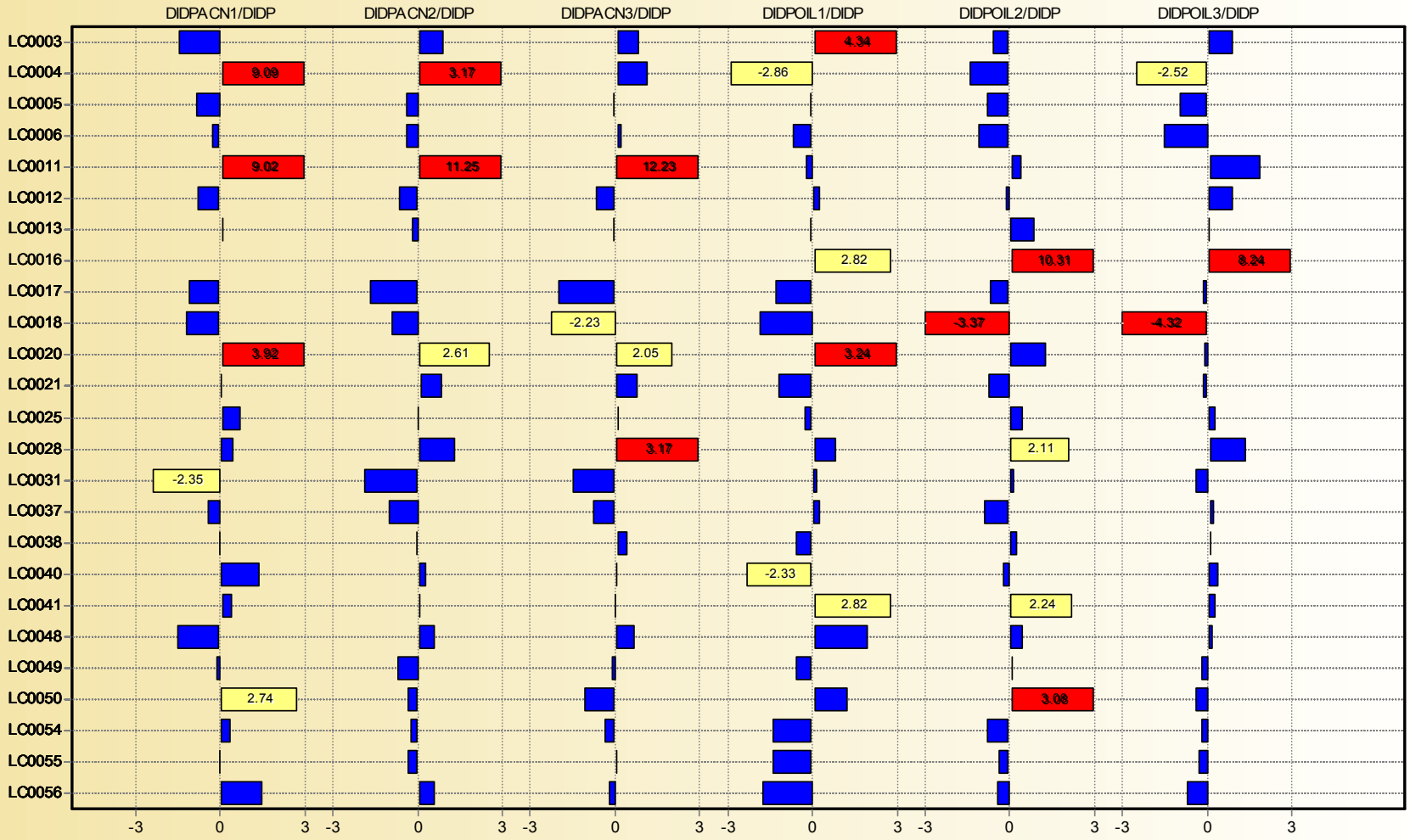
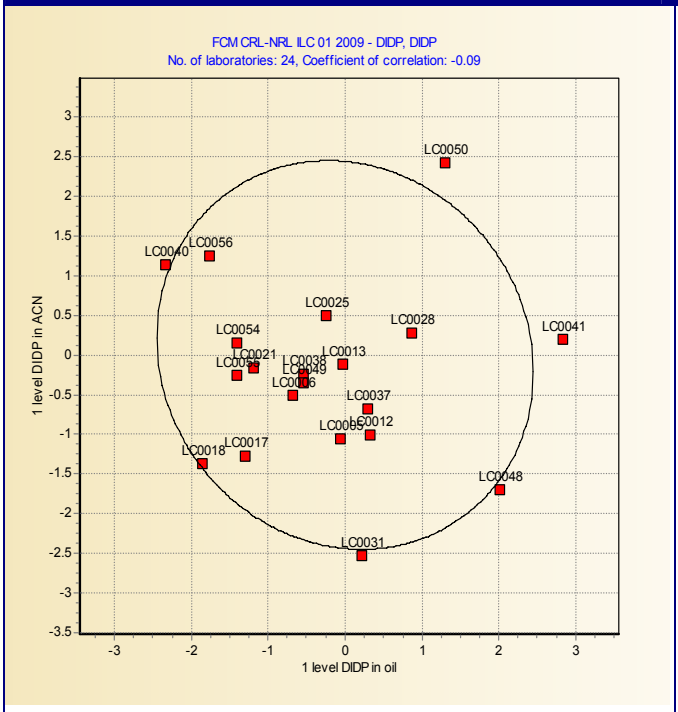
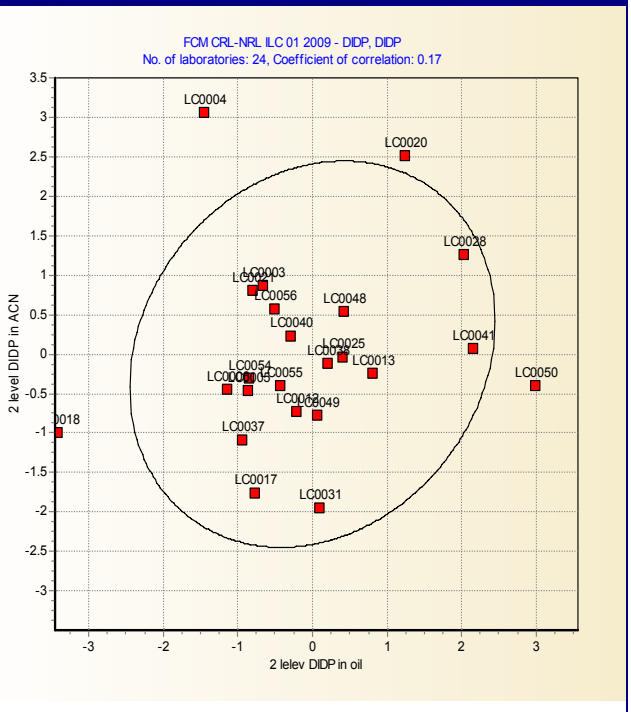


Figure 6. Youden plot

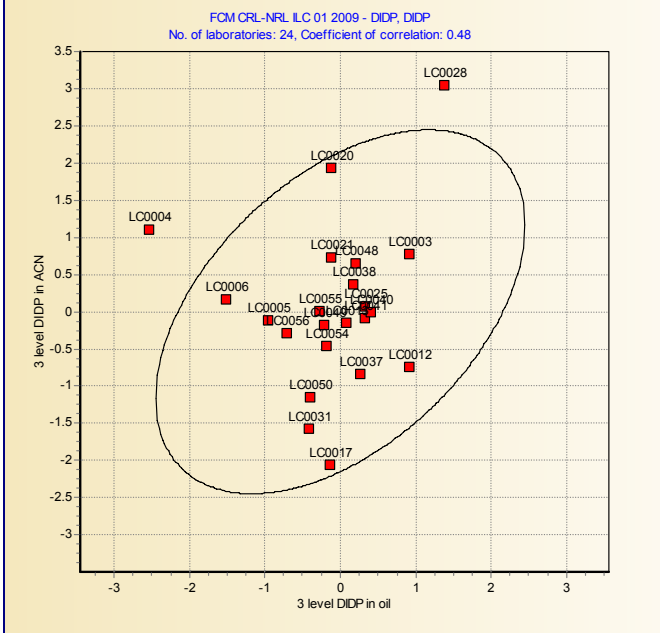
DIDP 1st level in ACN and in oil



DIDP 2nd level in ACN and in oil



DIDP 3rd level in ACN and in oil



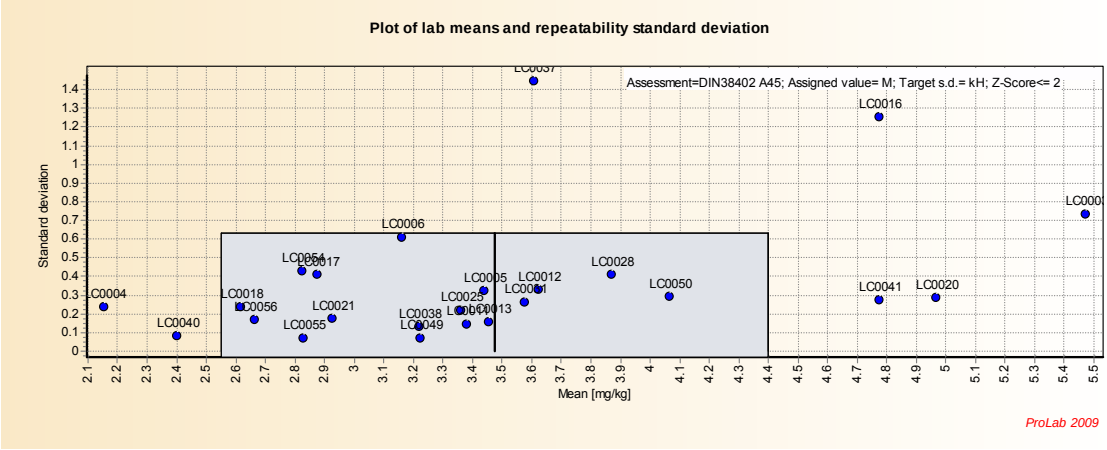
Results in the upper left or lower right hand corner of the diagram are dominated by random error.

Results close to the 45° line shown in the plot, but far away from the assigned value have results dominated by systematic error

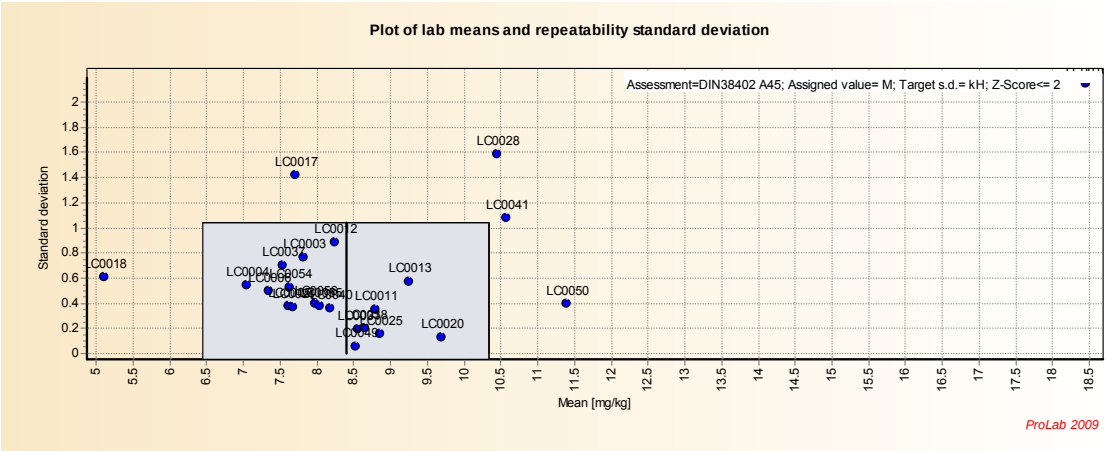
There is no correlation between the results of DIDP in oil and in because the correlation coefficient is less then 0.5-0.6

Figure 7 Laboratory mean values against its repeatability SD. Tolerance limits are calculated based on classical Horwitz SD.

a. DIDP in OIL 1st level



b. DIDP in OIL 2nd level



c. DIDP in OIL 3rd level

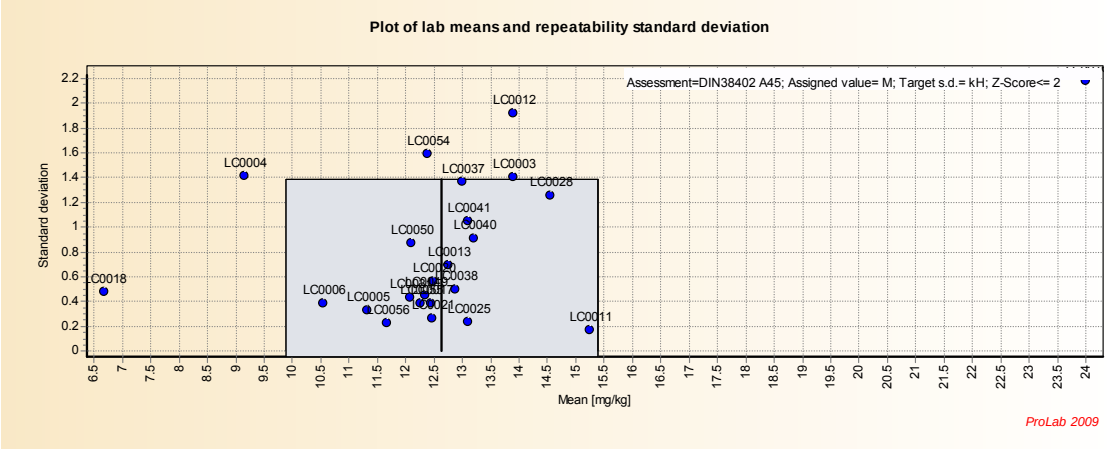
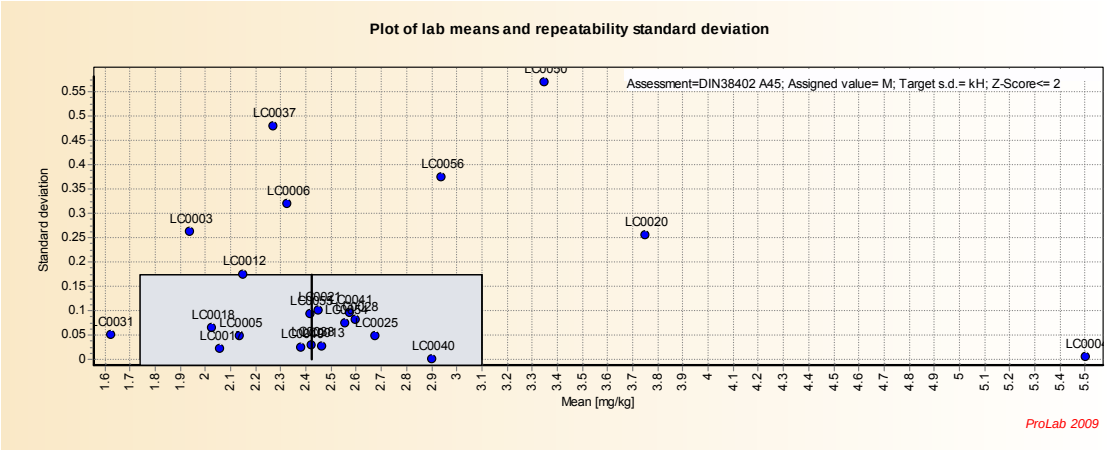
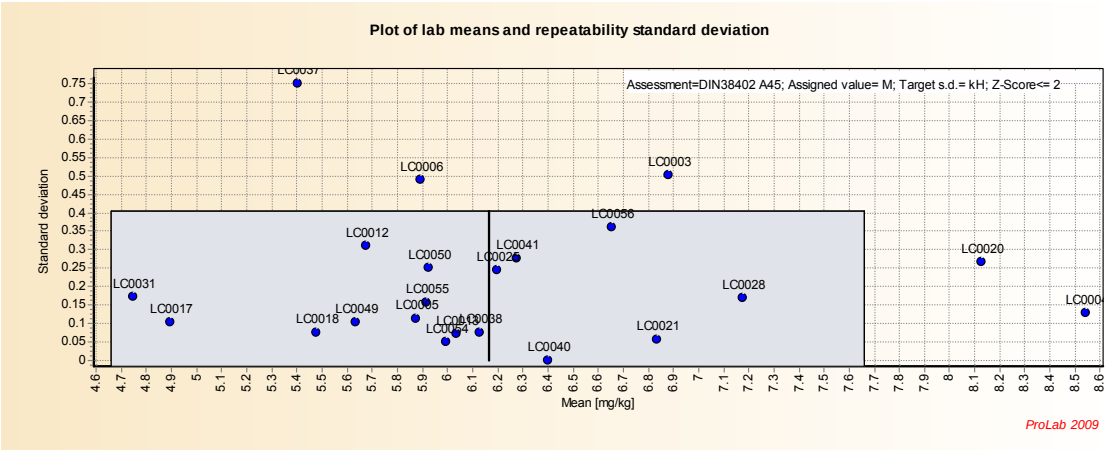


Figure 7 Laboratory mean values againds its repeatability SD. Tolerance limits are calculated based on classical Horwitz SD.

d. DIDP in ACN 1st level



e. DIDP in ACN – 2nd level



f. DIDP in ACN – 3rd level

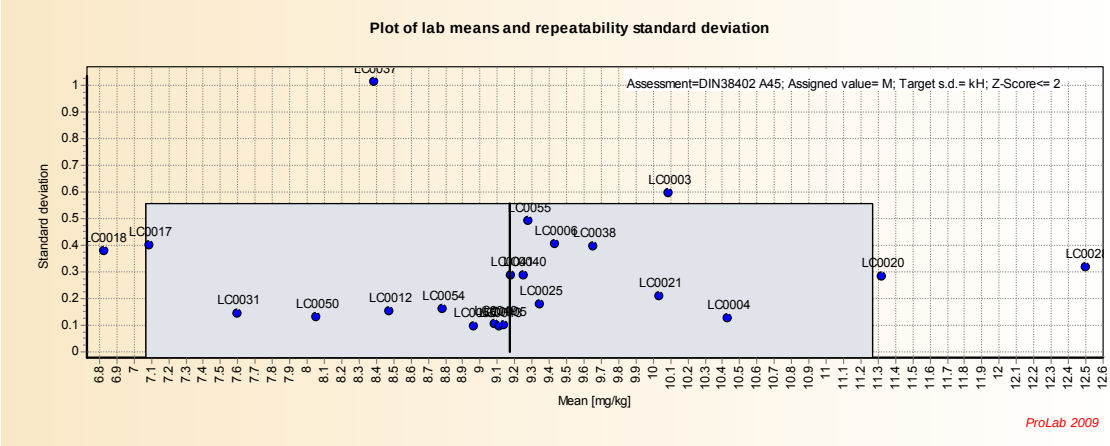
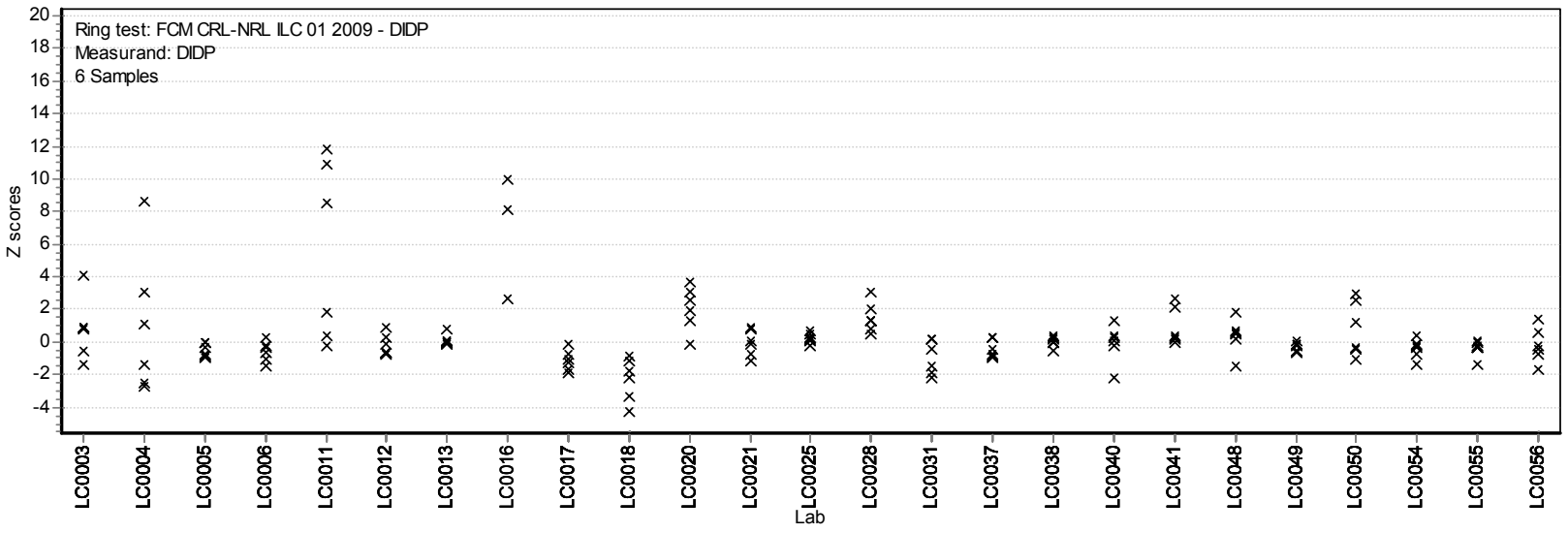


Figure 8:. Distribution of all z-score – scatter

Distribution of z scores

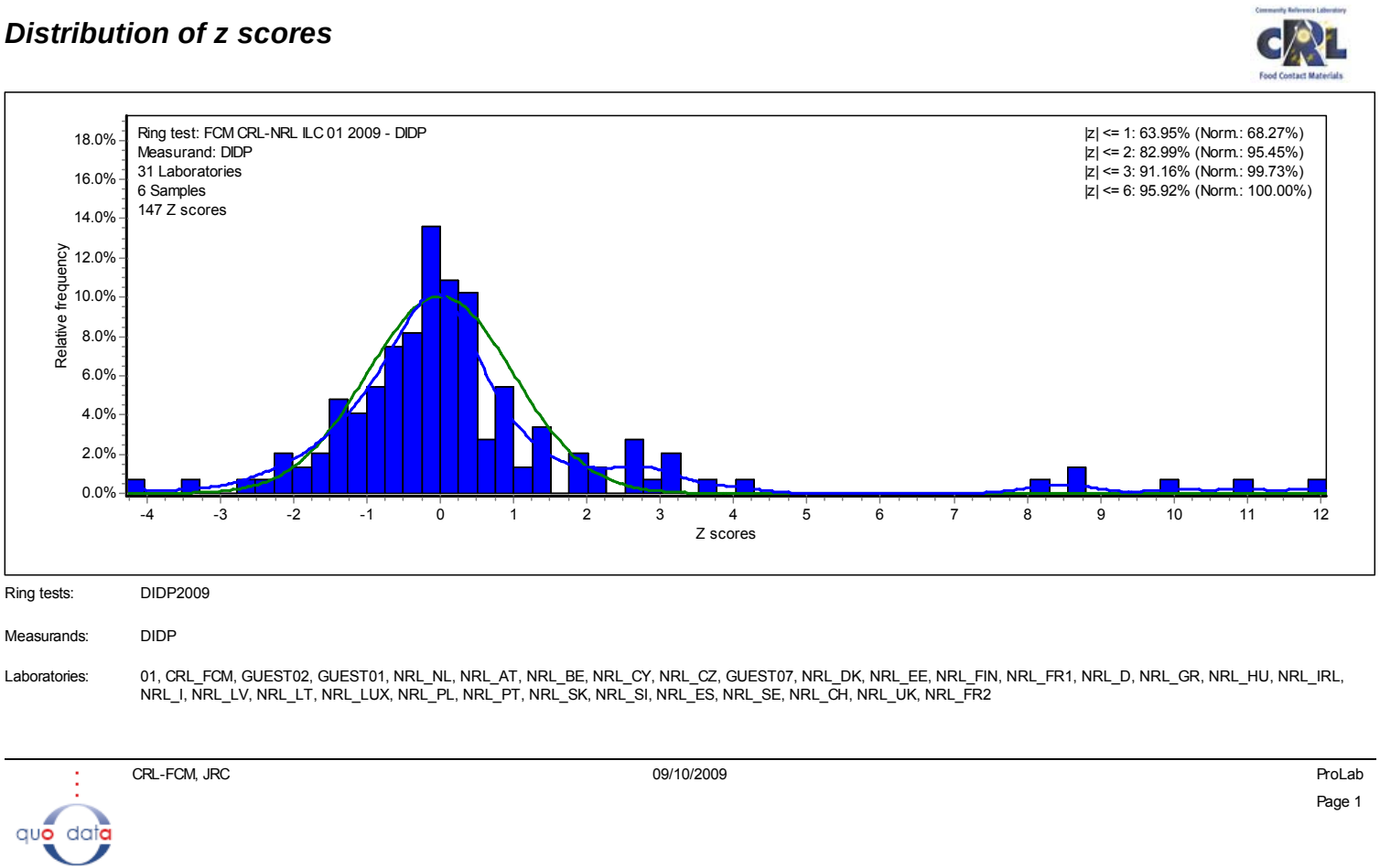


Ring tests: DIDP2009

Measurands: DIDP

Laboratories: 01, CRL_FCM, GUEST02, GUEST01, NRL_NL, NRL_AT, NRL_BE, NRL_CY, NRL_CZ, GUEST07, NRL_DK, NRL_EE, NRL_FIN, NRL_FR1, NRL_D, NRL_GR, NRL_HU, NRL_IRL, NRL_I, NRL_LV, NRL_LT, NRL_LUX, NRL_PL, NRL_PT, NRL_SK, NRL_SI, NRL_ES, NRL_SE, NRL_CH, NRL_UK, NRL_FR2

Figure 9:. Distribution of all z score histogram (blu bars), Kernel density plot (blue line) and normal distribution plot (green line)



9. Acknowledgements

The NRLs and guests participating in this exercise - listed below - are kindly acknowledged.

AUSTRIA	Austrian Agency for Health and Food Safety (AGES)
BELGIUM	Institute of Public Health, ISSP-LP
REPUBLIC OF CYPRUS	Laboratory for Control of Food Contact Materials and Control of Toys Ministry of Health, State General Laboratory (SGL)
CZECH REPUBLIC	NIPH- NRL for Food Contact Materials and for Articles for children under 3 years old, National Institute of Public Health (SZU')
DENMARK	Department of Food Chemistry, National Food Institute Technical University of Denmark
ESTONIA	Health Protection Inspectorate - Central Laboratory of Chemistry
FINLAND	Finnish Customs Laboratory
FRANCE	Center for Energy Material and Packaging - Laboratoire National d'Essais
FRANCE	SCL Laboratoire de Bordeaux-Pessac
GERMANY	Bundesinstitut für Risikobewertung (BFR) (Federal Institute for Risk Assessment)
GREECE	General Chemical State Laboratory, D' Chemical Service of Athens, Section Laboratory of Articles and Materials in Contact with Foodstuffs
HUNGARY	National Institute of Food Hygiene and Nutrition – Dept of Food additives and contaminants, Section Food Additives and Contact Materials
IRELAND	Public Analyst Laboratory - Sir Patrick Duns Hospital
ITALY	Istituto Superiore di Sanita', Laboratorio Esposizione e rischio da materiali, c/o Dipartimento ambiente e connessa prevenzione primaria
LATVIA	National Diagnostic Centre, Laboratory of Food and Environmental Investigations (LFEI)
LUXEMBOURG	Laboratoire National de Sante', Division du Controle des denrées alimentaires
POLAND	Laboratory of Department of Food and Consumer Articles Research , National Institute of Hygiene
PORTUGAL	ESB-SE (Portuguese Catholic University - Biotechnology College – Packaging Department)
SLOVENIA	National Institute of Public Health of Republic of Slovenia, Dept of Sanitary Chemistry
SPAIN	Centro Nacional de Alimentación, Agencia Espanola de Seguridad Alimentaria y Nutrición (AESAN)
THE NETHERLANDS	Food and Consumer Product Safety Authority (VWA), Inspectorate for Health Protection region North
Germany	Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Bavarian Health and Food Safety Authority
Germany	Central Institute of the German Armed Forces Medical Service Koblenz - Department III Food ChemistryHessisches Landeslabor LHL Standort Wiesbaden

10 References

[1] ISO 13528:2005; Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparisons

[2] M. Thompson, *Analyst*, (2000), 125, 385-386.

[3] (EC) No 372/2007 Commission Regulation of 2 April 2007 laying down transitional migration limits for plasticisers in gaskets in lids intended to come into contact with foods.

[4] 2002/72/EC Commission Directive of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs. (Plastics: Unofficial consolidated version including 2002/72/EC, 2004/1/EC, 2004/19/EC, 2005/79/EC, 2007/19/EC, 2008/39/EC)

[5] ProLab Software – QuoData, Drezden – www.quodata.de

[6] Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules

[7] DIN 38402 A45 Ringversuche zur externen Qualitätskontrolle von Laboratorien.

[8] ISO/TS 20612 Water quality – Interlaboratory comparison for proficiency testing of analytical chemistry laboratories

[9] T. Linsinger *et al.*, *Accreditation and Quality Assurance in Analytical Chemistry* (2001), 6, 20-25

[10] The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories by M. Thompson *et al.*, *Pure and Applied Chemistry* (2006), 78, 145–196

[11] ISO 5725-2:1994 (E) Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method

[12] AMC, *Representing data distributions with kernel density estimates*. AMC Technical Brief, 2006, http://www.rsc.org/images/brief4_tcm18-25925.pdf.

[13] Lowthian, P.J. and M. Thompson, *Bump-Hunting for the proficiency tester - searching for multimodality.* The Analyst, 2002. 127: p. 1359, https://www.swetswise.com/eAccess/viewAbstract.do?articleID=14625081.

[14] ISO 5725 -5 Accuracy (trueness and precision) of measurement method and results. Part 5: Alternative methods for the determination of the precision of a standard measurement method.

[15] ISO GUIDE 35:2006; Reference materials – General and statistical principles for certification.

[16] Horwitz & Albert, The Horwitz Ratio (HorRat): A useful Index of Method Performance with Respect to Precision, Journal of AOAC International, vol.89, No4, 2006

11. Annexes

Annex 1 Overview of the analytical methods used by the participants to the Comparative Trial 2008 PT001/B for the determination of DIDP in oil – summary of the information obtained from the questionnaire sent in February 2009 to NRL's and guests.

Annex 2 Standard Operating Procedure: Determination of Di-isodecyl Phthalate in Oil.

Annex 3: Invitation letter to laboratories ILC 01 2009

Annex 4: Format for confirmation of participation to ILC 01 2009.

Annex 5: Letter accompanying the samples ILC 01 2009.

Annex 6: Letters of confirmation of receipt ILC 01 2009

Annex 7: Instruction for the compilation of the results in electronic format.

Annex 8: Format for the compilation of results in non-electronic format.

Annex 9 Summary of laboratories participation in interlaboratory comparison exercises

Annex.10 Procedure for the preparation of the spike of DIDP in sunflower oil for ILC 01 2009

Annex 11: Results of the homogeneity study.

Annex 12: Results of the stability study.

Annex 1: Overview of the analytical methods used by the participants to the Comparative Trial 2008 PT001/B for the determination of DIDP in oil – summary of the information obtained from the questionnaire sent in February 2009 to NRLs and guests.

Sample preparation

Lab code	z score	How was the sub-sample obtained?	Mass of oil	Extraction solvent used	Extraction solvent volume	Extraction procedure	Sample work-up technique	Sample clean-up technique
LC0031	**0.05**	The oil was shaken prior to weighing into glass vials.	1g	Acetonitrile (ACN)	5mL	Vortex for 1 minute	Sample centrifuged for 5 min at ~3000 rpm	C18 SPE cartridge . Eluent is evaporated to dryness under N2 at 40°C and reconstitute in 1mL hexane.
LC0017	**0.18**	Sample was solved in DCM/ACN (6/4; v/v)	100 mg				none	None
LC0044	**-0.58**	The oil was shaken to mix and sub-samples were weighed into glass vials	5g	Acetonitrile	5 mL	Mixed on orbital shaker for 2 hours	Extracts centrifuged at 2000rpm for 5 minutes. Portion of acetonitrile layer removed for analysis.	None
LC0038	**0.91**		5 g	Hexane	5 mL	Mixing	5 g of oil spiked with IS and aliquot of 0.6 g dissolved with 5 mL of hexane	None
LC0005	**-1.08**	sub-samples weighed into glass vials	5 g	Acetonitrile	5 + 2 ml	Magnetic stirring during 6 hours	Extracts centrifuged at 2500 rpm for 5 minutes. Acetonitrile layer removed and evaporated under N2. Residue dissolved in 0.5 ml of acetonitrile for analysis.	None
LC0003	**-1.3**	The oil was shaken to mix and sub-samples were weighed into glass vials	5g	Organic solvents mixture		Mixed on orbital shaker for 5 min	Portion of organic solvents layer removed for analysis.	None
LC0013	**-1.46**	The oil was shaken to mix and sub-samples were weighed into 10 ml volumetric flasks	0.7 g	Dichloromethane	10 ml	None	Fill to the mark with dichloromethane and filter (0.45 um) prior its purification.	GPC. Collected fraction is evaporated to dryness and re-dissolved in acetonitrile.
LC0037	**-2.3**	The oil was shaken to mix and sub-samples were weighed into glass vials	5g	Acetonitrile	5 ml	Manual shake for 5 minutes		SPE (Vac Alum-N)
LC0049	**2.45**	The oil was shaken to mix and sub-samples were weighed into glass vials	1 g	acetonitrile/acetone	10 ml	Shaking (Vortex), centrifugation	Concentration, dilute in MTBE/ hexane (3+2)	
LC0028	**2.6**	The received samples were shaken and subsamples of oil was weighed into 10 ml measuring flasks	0.100 g	Ethyl acetate/ Cyclohexane 1:1 (oil is fully soluble in this mixture)	10 ml	None	None	GPC (Biobeads SX3)
LC0055	**-5.3**	The oil was shaken to mix and sub-samples were weighed into glass vials.	3 g	Acetonitrile (ACN)	2x5 mL	Mixed in vortex shaker at 800 rpm for 30 min	Extracts centrifuged at 5000 rpm for 10 minutes. Portion of acetonitrile layer removed for analysis. Then new portion of fresh ACN was added and extraction was repeated with the same volume of ACN.	None
LC0010	**23.75**	The oil was taken to mix and sub-samples were weighed into glass vials	0.5-1.0g	Dichloromethane	10 ml	Mixed well for 1 hour	Portion of prepared sample in DCM was taken for analysis	None
LC0018	**29**	The oil was shaken to mix and sub-samples were weighed into glass vials	0.5 g	Tested with hexane and tested with methanol	25 ml	Homogenisation and sonication	Extracts centrifuged at 2000rpm for 5 minutes.Portion of liquid of extraction layer removed for analysis.	None
LC0040	**not enough sensitivity**	Shaking the oil sample; 6 sub-samples are weighted into 10 ml volumetric glass flasks (after rinsing them with Toluol)	1 g	toluene	10 ml		Solving the oil sample in Toluene; 10% oil solution was used for GC-MS analysis)	None
LC0004	**no results**	The oil was shaken to mix and sub-samples wereweighed into glass vials	1g	Acetonitrile	5 ml	Vortex for 30sec and then by shaking on orbital shaker for 4 hours at room temperature	Allow the layers to separate. Portion of acetonitrile layer removed for analysis.	None
LC0048	**no results**							
	no results	aliquotation after mixing	200 – 300 mg	MeCN	3 ml	three times intensive shaking for at least 5 min	none	None

Annex 1 (continue): Overview of the analytical methods used by the participants to the Comparative Trial 2008 PT001/B for the determination of DIDP in oil – summary of the information obtained from the questionnaire sent in in February 2009 to NRLs and guests.

Analysis by gas chromatography (GC)

Lab code	z score	GC column - name phase dimensions	Injection volume	Injection mode	GC oven programme	Detector	MS – ions monitored	Ion used for quantification	Other (please provide details)
LC0031	**0.05**	DB-5MS, 5% Phenyl – 95 % Dimethylpolysiloxane, 60m length, 0.25mm I.D., 0.25µm film thickness	1µl	Splitless	60°C for 2 min, 13°C/min to 300°C held for 16 min	Varian 4000 MSD	307, 149 (153 for IS)	307 (153 for IS)	
LC0017	**0.18**								
LC0044	**-0.58**	Zebron ZB-5MS, 5% Polysilarylene – 95% Polymethylsiloxane 30m length, 0.25mm I.D., 0.25µm film thickness	1µl	Splitless	80°C for 1 min, 15°C/minute to 300°C held for 5 min. Carrier gas, Helium at 1mL/min.	Thermoquest Voyager Mass Spectrometer	307, 149, 207 (153 for IS)	307 (153 for IS)	
LC0038	**0.91**	HP-5MS, 5% Phenyl – 95 % Dimethylpolysiloxane, 30m length, 0.25mm I.D., 0.25µm film thickness	1µl	Splitless 280°C	100°C for 2min, 25°C/min to 180°C held for 1min, 10°C/min to 380°C held for 8 min	MS quadrupole	307	307	
LC0005	**-1.08**	VF-5MS, 5% Phenyl – 95 % Dimethylpolysiloxane, 30m length, 0.25mm I.D., 0.25µm film thickness	1.5 µl	splitless	60ºC for 1 min, 20ºC/minute to 220ºC, 5ºC/min to 300ºC held for 5 min. Carrier gas: Helium at 1 ml/min.	Varian 1200L Quadrupole MS	149 (153 for IS)	149 (153 for IS)	
LC0003	**-1.3**	FactorFour VF-5MS, 5% Polysilarylene – 95% Polymethylsiloxane, 30m length, 0.25mm I.D., 0.25µm film thickness	2µl	Split 1 :30	80°C for 0 min, 20°C/min to 180°C, held for 0 min, 6°C/min to 300°C held for 5 min	MS	307	307	
LC0013	**-1.46**								
LC0037	**-2.3**	HP5-MS, 5% Phenyl – 95 % Dimethylpolysiloxane, 30m length, 0.25mm I.D., 0.25µm film thickness	1µl	Splitless	80°C for 1min, 20°C/minute to 260°C held for 10 min, 20°C/minute to 280°C held for 10 min. Carrier gas, Helium at 1ml/min	Agilent 5973N	149	149	
LC0049	**2.45**	VF-5 MS, 5% Phenyl – 95 % Dimethylpolysiloxane, 30 m length, 0.25 mm I. D., 0.25 µm film thickness	1µl	Splitless, 270°C	80°C for 1 min, 10°C/minute to 300°C held for 7 minutes. Carrier gas: Helium at 1 ml/min	Varian 4000 MS (ion trap)	149 (115 + 157 for IS)	149 (115 + 157 for IS)	
LC0028	**2.6**	DB5-MS, 5% Phenyl – 95 % Dimethylpolysiloxane, 30m length, 0.25 mm I. D., 0.25 µm film thickness	100 µl	PTV	90ºC for 1 min, 50ºC/min to 200ºC, 15ºC/min to 300ºC held for 10 min	Agilent 5973 mass selective detector	149, 307 and 289 for DiDP and 153 and 297 for D4-DiDP (IS)	149 and 153	LVI-injections at 50º at 2,55 µl/sec. Hereafter 600ºC/min to 300 ºC kept in 10 min. 110 ml/min vent flow during injection.
LC0055	**-5.3**	HP-Ultra 1, 100 % dimethylpolysiloxane, 25m length, 0.32 mm I. D., 0.17 µm film thickness	1 µl	Splitless	70°C for 2.8 min, 15°C/min to 320°C held for 4 min. Carrier gas: constant flow of helium at 1.85 mL/min.	Agilent 5975C,inert XL EI/CI MSD with triple-axis detector	149, 307, 167, (249 for IS)	149 for both	
LC0010	**23.75**	VF – 5 MS, 5% Phenyl – 95 % Dimethylpolysiloxane, 30m lenght, 0.25mm I.D., 0.25µm film thickness	1µl	Splitless	50°C for 2 min, 10°C/min to 280 °C held for 5 min	Varian Saturn II Mass Spectrometer (IT)	149	149	
LC0018	**29**	VF - 5MS, 5% Phenyl – 95 % Dimethylpolysiloxane, 30m length, 0.25mm I.D., 0.25µm film thickness	1µl	Splitless	80°C for 2 min, 15°C/minute to 280°C held for 10 minutes. Carrier gas, Helium at 1mL/min.	Agilent Mass Spectrometer	307, 149 (115 for IS)	115 for IS	
LC0040	**not enough sensitivity**	TR-5MS, 5% phenyl polysilphenylene-siloxane -95%Polymethylsiloxane, 60m length, 0.25mm I.D., 0.25µm film thickness	1µl	Splitless; splitless time 2.1 min	90°C for 2.1 min, 15°C/min to 200°C , 5°C/min to 280°C held for 20 min. Carrier gas: Helium at 2ml/min constant flow	Finnigan Trace DSQ	149,307,167 (326,258 for IS)	307 for DIDP (326 for IS)	
LC0004	**no results**	HP-5 MS, 5% Phenyl – 95 % Dimethylpolysiloxane, 30m length, 0.25mm I.D., 0.25µm film thickness	1µl	Splitless	50°C for 1 min, 30°C/min to 280°C held for 20 min, 15°C/min to 300°C held for 3 min	Quatropole Mass Spectrometer	307, 149, 167	Only Qualitative Confirmation	
LC0048	**no results**	TR-50 MS, 50% phenyl polysilphenylene-siloxane, 30m length, 0.25mm I.D., 0.1 µm film thickness	1µl	Splitless	35°C for 0.50 min, 25°C/min to 300°C held for 13.9 min	Agilent GC-MS	307, 167, 149	307	
	no results	Zebron ZB-5MS, 5% Polysilarylene– 95% Polymethylsiloxane, 30m length, 0.25mm I.D., 0.25µm film thickness	1µl	splitless ; KAS 120°C – 320°C	80 – 320°C	Agilent 5973inert	307, 149	307	

Annex 1 (continue): Overview of the analytical methods used by the participants to the Comparative Trial 2008 PT001/B for the determination of DIDP in oil – summary of the information obtained from the questionnaire sent in February 2009 to NRLs and guests.

Analysis by liquid chromatography (LC)

Lab code	z score 2008	LC column - name packing dimensions	LC column temp.	Mobile phase	Flow rate	Inj. volume	Inj. mode	Detector	UV - wavelength	MS – ions monitored	Ion used for quantif.	MS/MS – transitions monitored	Transition used for quantify.
LC0031	**0.05**												
LC0017	**0.18**	Phenomenex Gemini NX, RP C18 110 A, 100x2mm, 3µm	40°C	A: ACN/H2O (9/1;v/v) B: EtOH (each 0,05% FAc)	0.4 mL/min	5 µL	Direct	Tandem MS				[M+H]+: 447-149; 447-	447-149
LC0044	**-0.58**												
LC0038	**0.91**												
LC0005	**-1.08**												
LC0003	**-1.3**												
LC0013	**-1.46**	Luna C18 (2)HST, C18, 50x3mm, 2.5µm	30ºC	H2O 0.1%AcOH / MeOH 0.1%AcOH (Gradient)	0.3 ml/min	10 µl	Parallel Fill mode	MS/MS Triple Quadrupole			447.3>140.9; 447.3>148.8	447.3>140.9	
LC0037	**-2.3**												
LC0049	**2.45**												
LC0028	**2.6**												
LC0055	**-5.3**												
LC0010	**23.75**												
LC0018	**29**												
LC0040	**not enough sensitivity**												
LC0004	**no results**	Phenosphere-Next 5u C8, 250x4.6m, 5µm	Room Temperature	Acetonitrile: Water (95:5)	Start with 1,5 ml/min and after 3 min continue with 2,0 ml/min	20µl		UV	254 nm				
LC0048	**no results**	Uplc BEH C18, 50x2.1mm, 1.7µm	30ºC	1% Hac: A CN	0.4 ml/min	2 µl	Partial loop	Tandem MS		447.3	85.1	447.3>85.1; 447.3>148.9	447.3>85.1
	no results												

Annex 1 (continue): Overview of the analytical methods used by the participants to the Comparative Trial 2008 PT001/B for the determination of DIDP in oil – summary of the information obtained from the questionnaire sent in February 2009 to NRL's and guests.

Other

Lab code	z score	Was a procedural blank sample prepared (i.e. no oil)	Was a background response observed?	If yes was the background response subtracted from the test result?	Was an internal standard (IS) used?	If yes which IS?	What concentration of IS was added?	Was an overspiked sample prepared?	If yes what concentration of DiDP was spiked?	If yes what was the recovery?	Was the reported concentration corrected for recovery?	How were the calibration standards prepared?	What was the calibration standard concentration range used?
LC0031	**0.05**	Yes	No		Yes	D4-DEHP	50 mg/kg in oil	2 overspiked samples of blank oil provided	11 mg/kg in oil	102%	Yes	Addition of test material to residue free sunflower oil	0 – 45 mg/kg in oil
LC0017	**0.18**	Yes, different kind of blank oil	No		Yes	D4-BBP		No				In blank olive oil	
LC0044	**-0.58**	Yes	No		Yes	D4-Benzybutyl phthalate	5 mg/kg in oil	2 overspiked samples	5 mg/kg in the oil	96%	Yes	Standard addition to blank oil provided with test materials	0-20 mg/kg in oil
LC0038	**0.91**	Yes	No		Yes	Dicyclo-hexyl phatalate (DCEP)	5 mg/kg	No				5 g of blank oil spiked with IS and compounds aliquots of 0.6 g dissolved with 5 mL of hexane	2 mg/kg – 14 mg/kg
LC0005	**-1.08**	Yes	No		YES	D4-Benzybutyl phthalate	4 mg/kg in oil	No				Standard addition of blank oil provided	0-25 mg/kg in oil
LC0003	**-1.3**	Yes	No		Yes	Diphenyl phthalate	30 mg/kg	No				Standard addition to blank oil provided with test materials	2.0 to 48 mg/kg
LC0013	**-1.46**	Yes	The oil-blank response was included in the calibration plot.		No			Yes	2.3 mg/Kg in oil	108.20%	No	Standard addition to blank oil provided with test materials after its purification.	0-9 mg/Kg in oil
LC0037	**-2.3**	Yes	Yes	Std Addition	Yes	Diethyl phthalate	0.4 mg/kg in oil	No			No	Standard addition to blank oil with test materials	4-7mg/Kg in oil
LC0049	**2.45**	No	No		Yes	Dimethyl pimelate	10 mg/kg in oil	Yes				Standard addition to blank oil provided with test materials	0-50 mg/kg
LC0028	**2.6**	Yes	No		Yes	3,4,5,6 ring D4-Di-n-Nonyl Phthalate	1,85 mg/kg	Yes	20 mg/kg	112.9%	No	In cyclohexane/ ethylacetate	0-22.5 mg/kg
LC0055	**-5.3**	Yes	No		Yes	Dicyclo-hexyl phthalate (DCP)	40 µg DCP/ml added to ACN before GC-MS analysis					2 blanks+3 calibration solutions in oil at 7, 14 and 21 mg/kg	0-21 mg/kg in oil
LC0010	**23.75**	Yes	No		No			No				Standards diluted in DCM	0-35 mg/kg in oil
LC0018	**29**	Yes	No		Yes	Dimethyl pimelate	100 mg/kg in oil					Standard addition to blank oil provided with test materials	0-25 mg/kg in oil
LC0040	**not enough sensitivity**	Yes, just solvent (Toluene) filled in 10 ml volumetric flask	Yes	Yes	Yes	PCB 97	200 mg/kg in oil	No				Solvent standard in Toluene; dilution of the solvent standard solution according to the levels from 2 to 50 mg/l	20-500 mg/kg in oil
LC0004	**no results**	Yes	No		No			No				External Std Calibration	12-48 mg/L in solvent
LC0048	**no results**												
	no results	blank oil was used	not for m/z 307		Yes	diallylphthalate	2 mg/l (final concentration in vial)	no (calibration procedure with spiked oil)				calibration procedure with spiked oil	5 – 25 ppm

Annex 2

DETERMINATION OF DI-ISODECYL PHTHALATE IN OIL

0 INTRODUCTION

Di-isodecyl phthalate (1,2 – Benzenedicarboxylic acid, di-isodecyl ester) is an plasticizer for plastics.

This SOP represents an analytical method for the determination of di-isodecyl phthalate into oil food simulant after migration from plastics.

1 SCOPE

This protocol describes a method for the determination of di-isodecyl phthalate in oil.

The method is appropriate for the quantitative determination of di-isodecyl phthalate in oil in approximate analyte concentration range of 1 to 20 mg/kg of oil.

2 PRINCIPLES

Di-isodecyl phthalate is extracted from oil with acetonitrile by shaking at room temperature. The organic phases are separated after centrifugation. Determination is carried out by means of gas chromatography-mass spectrometry (GC-MS). Quantification is achieved using an internal standard.

3 REAGENTS

3.1 Reference material, reagents and solvents

3.1.1. Di-isodecyl phthalate (DIDP), CAS 68515-49-1

- SIGMA ALDRICH, purity 99.8%

- JAYFLEX DIDP, ExxonMobil,

- DIPLAST R, Polynt

NOTE 1:

Profiles of DIDP from different suppliers are very close and are given as a reference in annex 1.
Purity is only stated for Sigma Aldrich DIDP (99.8%), but the difference in the slope of the regression lines obtained with the tree sources of DIDP is not significant

3.1.2. bis-(2-ethylhexyl)-phthalate-D4, CAS N 93951-87-2, purity > 98% - as internal standard

3.2.3. Acetonitrile (for UV grade or equivalent)

3.2.4. Hexane (AR grade or equivalent)

3.2 Solutions

3.2.1 Stock solution of di-isodecyl phthalate in hexane (1.0 mg/ml)

Weigh, to the nearest 0.1 mg, 100 mg di-isodecyl phthalate in a 100 ml volumetric flask. Fill volumetric flask up to the mark with hexane and mix. Calculate the exact concentration of the substance in mg/mL.

Note: The solution should be stored protected from light in a refrigerator (4-6ºC).

3.2.2 Intermediate standard solutions of di-isodecyl phthalate in hexane (0.1 mg/ml)

Pipette 1.0 ml of the standard stock solution into a 10 ml volumetric flask and fill the flask up to the mark with r hexane. Calculate the exact concentration of di-isodecyl phthalate in µg/ml.

3.2.3 Stock solution of bis-(2-ethylhexyl)-phthalate-D4 (D4-DEHP) in hexane (1.0 mg/ml).

Weigh, to the nearest 0.1 mg, 100 mg of bis-(2-ethylhexyl)-phthalate-D4 into a 100 mL volumetric flask. Fill volumetric flask up to the mark with hexane. Calculate the exact concentrations of the substances in mg/ml.

Note: The solution should be stored protected from light in a refrigerator (4 - 6ºC).

4 Laboratory equipment

4.1 Calibrated balance accurate to 0.01 g

4.2 Calibrated balance accurate to 0.01 mg

4.3 Centrifuge able to reach 2500 rpm.

4.4 Block heater with nitrogen gas supply

4.5 Digital syringes or pipettes, 25, 250 ul

4.6 24 ml clear glass screw cap vials

4.7 Normal laboratory glassware and apparatus

5 GC-MS apparatus

5.1 Gas chromatograph equipped with an autosampler and in connection with mass selective detector.

5.2 GC column, capable of delivering reproducible peaks of di-isodecyl phthalate and bis-(2-ethylhexyl)-phthalate-D4 as an internal standard, and capable to separate this peaks from interference peaks originated from samples used.

NOTE 2:

For guidance, the instrument parameters which are found suitable for the analysis, using the selected column are given in Annex 2.

6 PROCEDURE

6.1 Test sample preparation

- Weight 1 g of oil in 24 ml clear screw cap vials;
- Add 10 µl of the 1.00 mg/ml internal standard solution (3.2.3);
- Add 10 ml of acetonitrile, close the vial and shake manually for a while;

NOTE 3:

Tests have been carried out with different oil:ACN ratio starting from 1:1 up to 1:20 in order to increase the absolute recovery of DIDP in ACN, due to the low partitioning coefficient of DIDP. Ratio 1:10 could be regarded as acceptable

- Shake on Vortrex shaker for 2 minute.

NOTE 4:

Tests have been carried out with different way of mixing – Vortrex for 2 min, mechanical shaking for 2h, and mechanical shaking for 24h - resulting in no significant difference in the recovery rates.

- Centrifuge to separate the phases for 5 minutes at approx. 2500 rpm (1260 rcf);

NOTE 5:

The centrifuge speed is not critical parameter. The only requirement is to separate the oil and ACN phases..

- Transfer the acetonitrile phase with Pasteur pipette to a separate 24 ml vial .
- Reduce the volume of the acetonitrile on a block heater (4.4) set to 40°C with a gentle stream of nitrogen to approximate 1 ml.

NOTE 6:

Tests have been carried out for reducing the volume of ACN from 10 to 1 ml at ambient temperature and at 40°C for speeding up the procedure - resulting in no significant difference in the recovery rates.

- Leave the vial for approx. an hour for better separation of the remaining oil from acetonitrile and then transfer an aliquot from the top layer to a crimp cap vial for GC analysis and proceed as in 6.3.

NOTE 7:

In case when there is no good phase separation proceed with second centrifugation of the 1 ml ACN phase. No statistical significant difference was observed in the accuracy of the method. We do not recommend chilling or filtration.

6.2 Preparation of the calibration curve for GC-MS analysis

- Weight 1 g of oil in 24 ml clear screw cap vials;
- Add corresponding quantity (see the table below) of DIDP intermediate standard solutions (3.2.2)
- Add 10 µl of the the 1000 µg/ml internal standard solution (3.2.3)
- Add 10 ml of acetonitrile, close the vial and shake manually for a while
- Shake on Vortrex shaker for 2 minute
- Centrifuge to separate the phases for 5 minutes at 2500 rpm (1258 rcf) (note 5)
- Transfer the acetonitrile phase with Pasteur pipette to a separate 24 ml vial .
- Reduce the volume of the acetonitrile on a block heater (4.4) set to 40°C with a gentle stream of nitrogen to approximate 1 ml.
- Leave the vial for approx. an hour for better separation of the remaining oil from acetonitrile and then transfer and aliquot to a crimp cap vial for GC analysis and proceed as in 6.3.

Concentration level in oil [mg/kg]	Oil [g]	Spiking volume [µl]	Intermediate stock solution for DIDP [µg/ml]	Volume of IS (D4-DEHP) solution [µl]
0	1	0	100	10
1	1	10	100	10
4	1	40	100	10
8	1	80	100	10
12	1	120	100	10
16	1	160	100	10
20	1	200	100	10

6.3. GC-MS analysis

Before starting measurements, examine the base line stability and response linearity.

Maintain the same operating conditions of the GC-MS system throughout the measurements of all samples and calibration solutions.

NOTE 8:

For guidance, the instrument parameters which are found suitable for the analysis, using the selected column are given in Annex 2.

7. Calibration

7.1. Analysis of calibration standard solutions

Inject the relevant calibration solutions (6.2). Integrate peaks and measure peak area for di-isodecyl phthalate and bis-(2-ethylhexyl)-phthalate-D4 (internal standard). Construct a calibration function by plotting the amount ratio against the response ratio of the analytes and the internal standards in the calibration solutions. Calculate the regression parameters, correlation coefficient.

The calibration curve shall be linear and the correlation coefficient shall be 0,996 or better. If either of the two requirements is not met, fresh standard solutions shall be prepared from the original standard solutions. Analysis of the solutions and construction of the calibration graph have to be repeated.

NOTE 9:

Parameters of calibration curve are included in annex 3.

7.2 Analysis of samples

Inject the sample solutions prepared in 6.1. under the same conditions used for the calibration solutions. Observe the chromatogram and compare the retention time of peaks with the retention time obtained for the reference substances di-isodecyl phthalate and bis-(2-ethylhexyl)-phthalate-D4 as IS in 6.1

Measure the peaks area of di-isodecyl phthalate and bis-(2-ethylhexyl)-phthalate-D4 as IS for calculation of the DIDP concentration against established in 6.1 regression. Internal standard is used to compensate for the losses of analytes caused by sample handling, adsorption effects etc.

NOTE 10:

Check the chromatograms for peaks that might disturb the analysis of the target compounds

7.3 Evaluation of data

7.3.1 GC-MS interferences

No interferences should be detected. However, if the GC-MS chromatogram of the blank sample solution shows an interfering peak in the region of the analytes or internal standard, then different chromatographic conditions and/or an alternative extraction solvent shall be used.

7.3.2 Calculation of the di-isodecyl phthalate in the oil sample in mg/kg .

$$\text{Concentration DIDP in oil (mg/kg)} = \frac{C}{W}$$

where;

C is the plasticizer mass in µg derived from the calibration curve, and
W is the weight of sample taken in g.

8 CONFIRMATION

For confirmation of peak identity the retention time and a ratio between qualifier ions shall be taken into account.

Annex 1

Fig. 1. GS-MS scan chromatograms of DIDP from the different suppliers

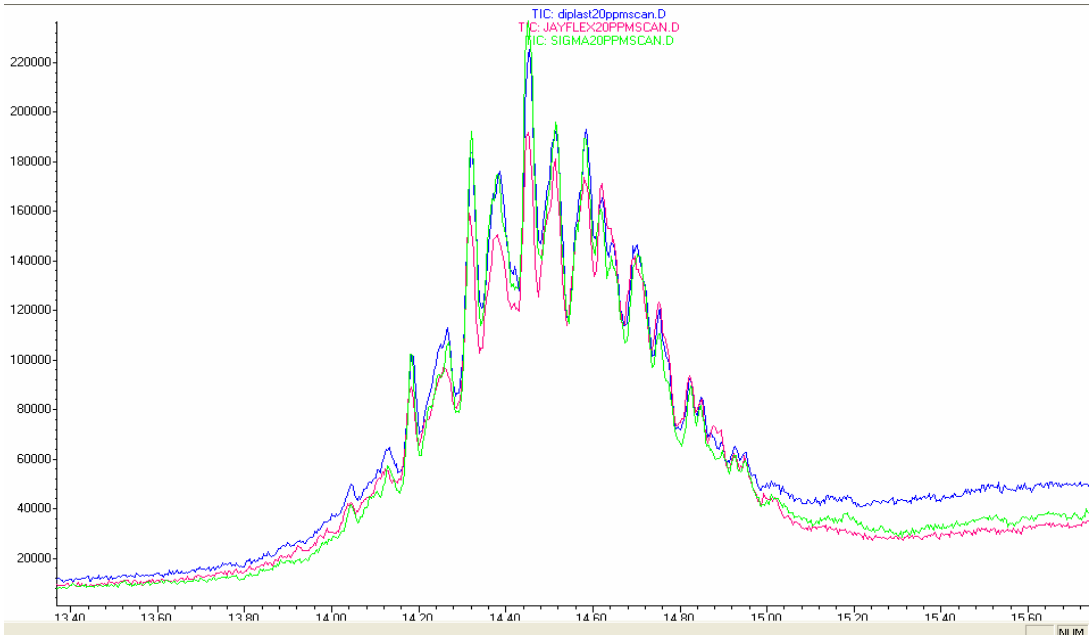


Fig. 2. GS-MS SIM (307) chromatograms of DIDP from the different suppliers

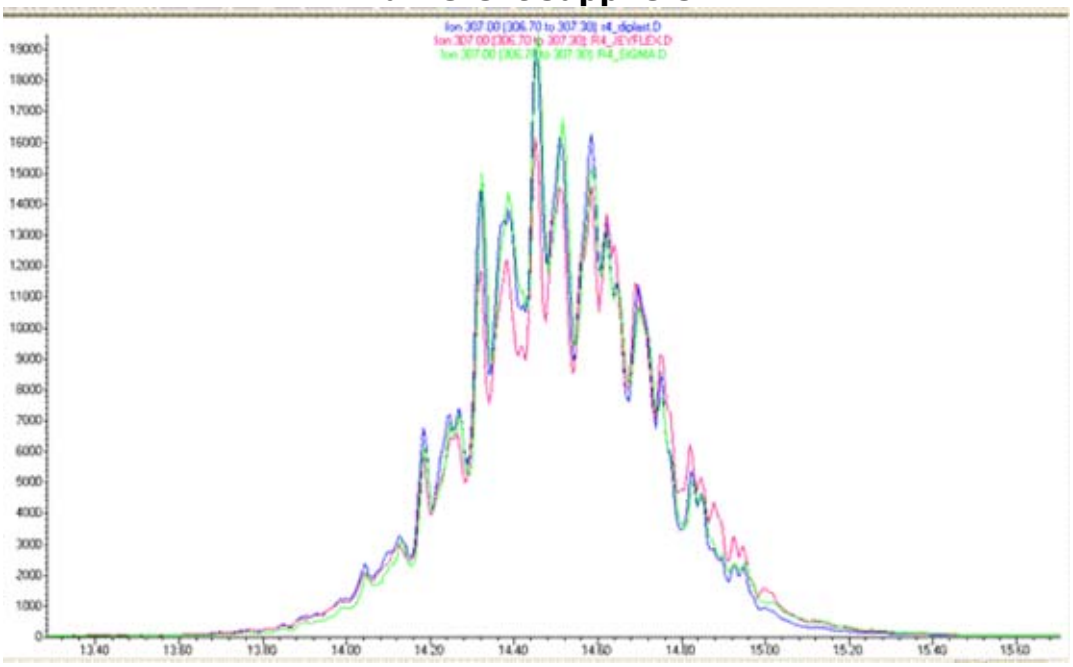
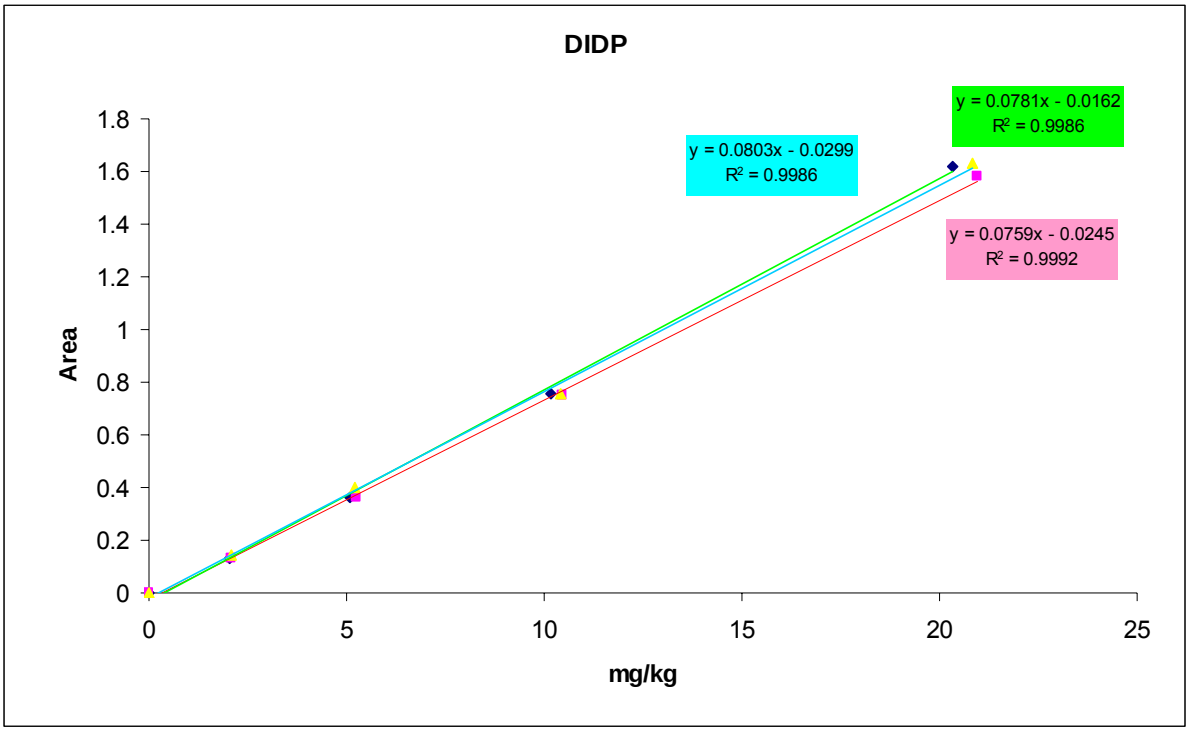


Fig. 3. Regression parameters for calibration curve of DIDP from the different suppliers *(EURL_FCM)*

- SIGMA ALDRICH, purity 99.8%

- JAYFLEX DIDP, ExxonMobil,

- DIPLAST R, Polynt



Annex 2

GC-MS Conditions (for information)

1. GC conditions

System	Agilent 5980 GC or equivalent
Analytical Column:	5% phenyl, 95% polymethylsyloxane, 30m x 0,25mm I.D. x 0,10 µm film thickness
Oven temperature program:	80°C, 1 min → 15°C/min → 300°C, 5 min
Carrier gas	Helium
Flow rate	1.3 ml/min
Injector temperature:	280°C
Injection mode:	splitless
Injection volume:	1µl

2. MS conditions

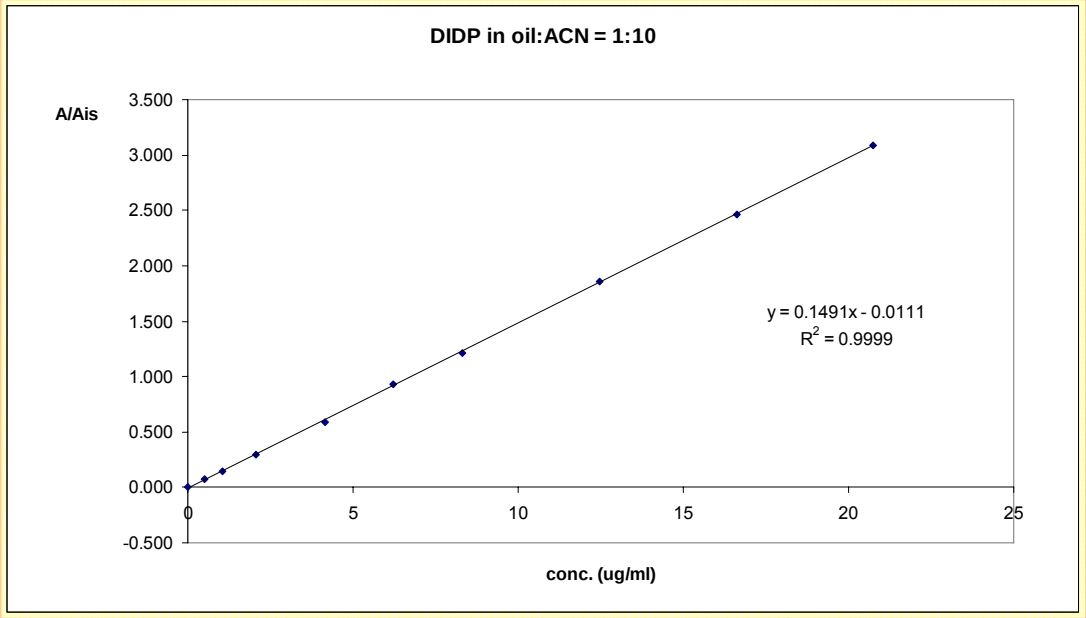
Mass-selective detector	Agilent 5973 MSD or equivalent - quadropole analyser
Transfer line temperature	320°C
Source temperature	230°C
Filament delay	4 minutes
Detection:	SIM mode

	Target ion	**Qualifier ion**	**RT (minutes)**
Di-isodecyl phthalate (DIDP)	307	149	13.6 -15.9 min
D4-DEHP	283	153	12.6 min

Annex 3

Parameters of calibration curve for di-isodecyl phthalate and detection limit

Parameters of calibration curve	Di-isodecyl phthalate in oil
Slope	0.1491
Intercept	- 0.0111
Correlation coefficient	0.9999
Range	1.0 - 20 mg/kg
Detection limit (approx.)	0.4 mg/kg
Determination limit (approx.)	1.2 mg/kg



Annex 3: Invitation letter to laboratories ILC 01 2009

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Unit Chemical Assessment and Testing

Community Reference Laboratory
Food Contact Materials

Ispra May 25, 2009
I02-CAT/CS/sm(2009)

Dear Madam, Sir

Comparative trial 2009 ILC 2009 - 01 from CRL FOOD CONTACT MATERIALS
"Analysis of DIDP in oil and solvent"

On behalf of the CRL for food contact materials, I would like to invite you to participate in a comparative trial/interlaboratory comparison (ILC) exercise for the determination of DIDP in oil and solvent which is due to start in the next weeks. Please note that according to the agreement of the December's CRL-NRL FCM plenary, this year the ILC exercise is a validation study of the method for the determination of DIDP in oil. Each participant will be asked to follow strictly the method description (SOP) agreed to (by written consultation of 2nd April) which will be provided in the kit.

I would like to remind you that it is a duty for you as an NRL-FCM to participate in the ILCs organised by the CRL-FCM since the work programme is decided with your agreement. For this reason we encourage all of you to actively participate in this exercise. There is no charge for participation. Feel free to involve your local controls.

We have pre-registered everyone, which means we will send test kits to all of you. We however need to receive the **proformat of your participation** for our own administrative purposes. Kindly send back the proformat **by June 05** to: Catherine Simoneau (catherine.simoneau@jrc.ec.europa.eu)**.** If you need more test kits to involve more laboratories at the national level we have another 20 kits of test materials for DIDP in oil and solvent. In this case please let me know immediately by e-mail so we can pack accordingly.

The samples will be sent to you in the second half of June. You will find additional information in the kit sent and on the form "shipment test DIDP". You will also receive more detailed instructions for the compilation of the results. The deadline for submission of results is **30th July 2008.**

If you have any question, plase contact Catherine Simoneau (catherine.simoneau@jrc.ec.europa.eu), ph. +39.0332.785889

Sincerely yours,

Catherine Simoneau

Dr. Catherine Simoneau
Operating Manager, European Union Reference Laboratory for Food Contact Materials
European Commission, DG-Joint Research Centre
Institute for Health and Consumer Protection
Unit Physical and Chemical Exposure, T.P. 260
Ispra Va 21020 Italy

Cc: MM. D. Kotzias (JRC), D. Sarigiannis (JRC), B. Larsen (JRC), F. d'Atri (SANCO)
Mrs. A Schaefer (SANCO)

Direct access EURL: ph: +39.0332.785889 Fax: +39.0332.785707 e-mail: catherine.simoneau@jrc.it http://EURL-fcm.jrc.it/

Annex 4: Format for confirmation of participation to ILC 01 2009.

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Unit Chemical Assessment and Testing

Community Reference Laboratory
Food Contact Materials

Ispra May 25, 2009
Annex to I02-CAT/CS/sm(2009)

Participation to CRL-FCM ILC 2009 - 01
Interlaboratory comparison (ILC) exercise
for the determination of DIDP in oil and solvent.

CONFIRMATION OF PARTICIPATION

Your Name:	
Organization:	
Address:	
E-mail:	
Phone:	

item	YES	NO
I will participate the collaborative trial on analysis of DIDP in oil and solvents and will deliver results on time		
I have already the package with the files for filling the results and especially RingDat3.exe file from last year and I need only lab files for this year's ILC		
I do not have the package with the files for filling the results from last year		

Kindly send back this proformat to: Catherine Simoneau (catherine.simoneau@jrc.it) **by June 09.**
The samples will be sent to you in the second half of June. You will find additional information in the kit sent. The deadline for submission of results is **30th July 2009**

If you have any question, please contact Catherine Simoneau (catherine.simoneau@jrc.it), ph. +39.0332.785889

Sincerely yours,

Catherine Simoneau

Direct access EURL: ph: +39.0332.785889 Fax: +39.0332.785707 e-mail: catherine.simoneau@jrc.it http://EURL-fcm.jrc.it/

Annex 5: Letter accompanying the sample ILC 01 2009.

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Unit Chemical Assessment and Testing

Community Reference Laboratory
Food Contact Materials

Ispra June 11, 2009
Appendix to I02-CAT/CS/sm(2009)

Shipping kit for interlaboratory comparative testing CRL-FCM
ILC01 2009 – DIDP in oil and solvent

Standard substances kit:

- **DEHP- D4** - 10 mg di-ethylhexyl phthalate to be used as internal standard, *will be shipped separately due to delay in delivery from the supplier*

Shipping kit - DIDP in:

- **acetonitrile (ACN) -** three 12 ml screw cap vials containing about 10 ml of acetonitrile spiked with DIDP at 3 concentration levels.

- **sunflower oil (OIL) -** four 100 ml brown crimp cap bottles containing about 50 ml of oil spiked with DIDP at 3 concentration levels and blank oil.

Documentation:

- instruction for the compilation of results;
- letters of confirmation of receipt ILC 01 2009;
- empty form "Test results" for filling the results;
- method of analysis - SOP for DIDP in oil;

Results requested:

result format: Use the personal "NAME.LAB" file that will be provided by e-mail;

use the printed empty form "Test results" in case of difficulties with the files.

Direct access EURL: ph: +39.0332.785889 Fax: +39.0332.785707 e-mail: catherine.simoneau@jrc.it http://EURL-fcm.jrc.it/

Annex 6: Instructions for the compilation of the results in electronic format.

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Unit Chemical Assessment and Testing

Community Reference Laboratory
Food Contact Materials

Ispra June 11, 2009
Appendix to I02-CAT/CS/sm(2009)

Instructions for the compilation of the results for interlaboratory comparative testing CRL-FCM ILC01 2009

DEADLINE: Friday, July 31st

Results requested:
Perform four replicates for each sample and report all the four replicate data using the unit of measure specified in the lab file or printed paper "Test results":

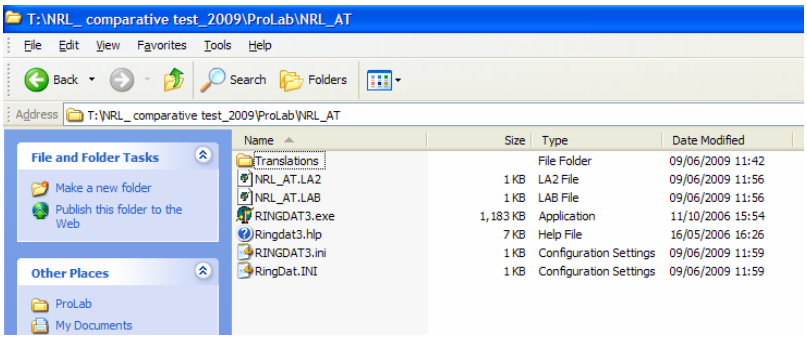
Compilation of results
Data generated by the laboratories for the comparative test CRL-FCM PT001 will be processed by the CRL-FCM using a software package for statistical analyses and professional data handling of interlaboratory tests.

- a simple data entry program (RingDat3.exe) is provided to each participating laboratory (from last year PT 2008 or now on CD-ROM 2009) ;
- two additional lab files with the extension "participant.Lab" and "participant.LA2", generated by the ProLab software are provided to each laboratory individually (personal files) by e-mail;
- the name of each laboratory and the samples are codified by the software, so that each participant will receive a sample with unique codified numbers (i.e., 0586);
- The "*.LA2" file contains information about the participant – laboratory name and laboratory code;
- The "*.LAB" file is unique to each laboratory (personal) and contains information about the samples (samples code) and measurands that have to be analyzed and reported.

Each laboratory has to start the RingDat3.exe program and to open "name.LAB" file for reporting the results. A table will appear with cells for every measurand/sample combination. That is why 6 sample codes will appear for each participant/file - 3 for acetonitrile and 3 for oil samples.

Procedure to be followed by laboratories for the opening the LAB files from the software.

First: Create a folder on your computer and transfer RingDat files and Translation directory (from last year PT2008 or from CD-ROM 2009) into it. Copy there as well the "NRL_X.LAB" and "NRL_X".LA2 files which you'll receive by mail. The folder should contain:



Direct access EURL: ph: +39.0332.785889 Fax: +39.0332.785707 e-mail: catherine.simoneau@jrc.it http://EURL-fcm.jrc.it/

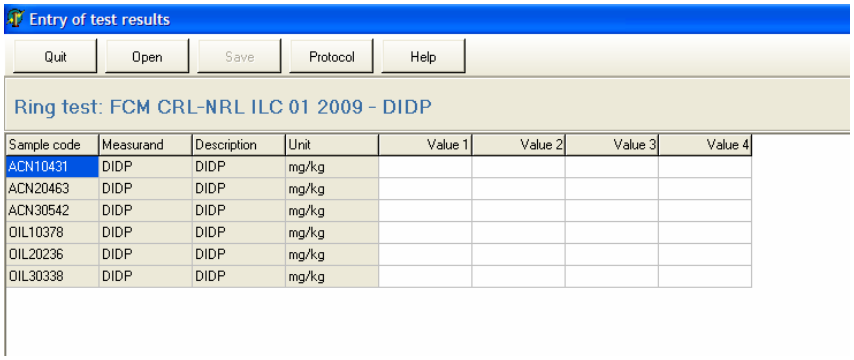
EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Unit Chemical Assessment and Testing

Community Reference Laboratory
Food Contact Materials

Then:

- Open the file "RINGDAT3.exe"
- Click on "Open" command
- Select the "NRL_X.LAB" file (where X is the member state abbreviation- with one to three letters) and click on "Open" command using the button on the top menu of the window

- Windows you should see using the software is :



Sample code	Measurand	Description	Unit	Value 1	Value 2	Value 3	Value 4
ACN10431	DIDP	DIDP	mg/kg				
ACN20463	DIDP	DIDP	mg/kg				
ACN30542	DIDP	DIDP	mg/kg				
OIL10378	DIDP	DIDP	mg/kg				
OIL20236	DIDP	DIDP	mg/kg				
OIL30338	DIDP	DIDP	mg/kg				

- Fill the table with your data
- Save the file using the button on the top menu of the window
- Send only the "NRL_X.LAB" file by e-mail to catherine.simoneau@jrc.it

Please fill your results and send it back by e-mail to Catherine Simoneau (catherine.simoneau@jrc.it) by 31th July 2009.

If you have any question, please contact Catherine Simoneau (catherine.simoneau@jrc.it), ph. +39.0332.785889

Sincerely yours,

Catherine Simoneau
Dr. Catherine Simoneau
Operating Manager, European Union Reference Laboratory for Food Contact Materials
European Commission, DG-Joint Research Centre
Institute for Health and Consumer Protection
Unit Physical and Chemical Exposure, T.P. 260
Ispra Va 21020 Italy

Direct access EURL: ph: +39.0332.785889 Fax: +39.0332.785707 e-mail: catherine.simoneau@jrc.it http://EURL-fcm.jrc.it/

Annex 7: Form for the compilation of the results in non-electronic format.

Ring test: FCM CRL-NRL ILC 01 2009 - DIDP

Test results

Lab.-code: LC0021

Sample code	Measurand	Description	Unit	Value 1	Value 2	Value 3	Value 4
ACN10351	DIDP	DIDP	mg/kg				
ACN20581	DIDP	DIDP	mg/kg				
ACN30456	DIDP	DIDP	mg/kg				
OIL10196	DIDP	DIDP	mg/kg				
OIL20129	DIDP	DIDP	mg/kg				
OIL30337	DIDP	DIDP	mg/kg				

..... Place and date

..... Manager of laboratory (in block letters)

..... Signature

Annex 8: Letter of confirmation of receipt of ILC 2009/01

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Unit Chemical Assessment and Testing

Community Reference Laboratory
Food Contact Materials

Ispra June 11, 2009
Appendix to I02-CAT/CS/sm(2009)

PARTICIPATION TO CRL-FCM ILC01 2009
DIDP IN OIL AND SOLVENT

CONFIRMATION OF RECEIPT OF THE SAMPLES

Please return this form to confirm that the sample package has arrived. In case the package is damaged, please state this on the form and contact us immediately.

Your Name:	
Organization:	
Address:	
E-mail:	
Phone:	

Any remarks ……..

Date arrival package ……..

Signature ……..

Kindly send back this form to: Catherine Simoneau (catherine.simoneau@jrc.it).

Sincerely yours,

Catherine Simoneau

Dr. Catherine Simoneau
Operating Manager, European Union Reference Laboratory for Food Contact Materials
European Commission, DG-Joint Research Centre
Institute for Health and Consumer Protection
Unit Physical and Chemical Exposure, T.P. 260
Ispra Va 21020 Italy

Annex 9 Summary of laboratories participation in interlaboratory comparison exercises

Member State	Name of NRL who participated in the ILC
AUSTRIA	Austrian Agency for Health and Food Safety (AGES),
BELGIUM	Institute of Public Health, ISSP-LP
REPUBLIC OF CYPRUS	Laboratory for Control of Food Contact Materials and Control of Toys Ministry of Health, State General Laboratory (SGL)
CZECH REPUBLIC	NIPH- NRL for Food Contact Materials and for Articles for children under 3 years old, National Institute of Public Health (SZU')
DENMARK	Department of Food Chemistry, National Food Institute Technical University of Denmark
ESTONIA	Health Protection Inspectorate - Central Laboratory of Chemistry
FINLAND	Finnish Customs Laboratory
FRANCE	Center for Energy Material and Packaging - Laboratoire National d'Essais SCL Laboratoire de Bordeaux-Pessac
GERMANY	Bundesinstitut für Risikobewertung (BFR) (Federal Institute for Risk Assessment) + 2 laboratories guests
GREECE	General Chemical State Laboratory, D' Chemical Service of Athens, Section, Laboratory of Articles and Materials in Contact with Foodstuffs
HUNGARY	National Institute of Food Hygiene and Nutrition – Dept of Food additives and contaminants, Section Food Additives and Contact Materials
IRELAND	Public Analyst Laboratory - Sir Patrick Duns Hospital
ITALY	Istituto Superiore di Sanita', Laboratorio Esposizione e rischio da materiali, c/o Dipartimento ambiente e connessa prevenzione primaria
LATVIA	National Diagnostic Centre, Laboratory of Food and Environmental Investigations (LFEI)
LUXEMBOURG	Laboratoire National de Sante', Division du Controle des denrées alimentaires
POLAND	Laboratory of Department of Food and Consumer Articles Research , National Institute of Hygiene,
PORTUGAL	ESB-SE (Portuguese Catholic University - Biotechnology College – Packaging Department)
SLOVENIA	National Institute of Public Health of Republic of Slovenia , Dept of Sanitary Chemistry,
SPAIN	Centro Nacional de Alimentación, Agencia Espanola de Seguridad Alimentaria y Nutrición (AESAN)
THE NETHERLANDS	Food and Consumer Product Safety Authority (VWA), Inspectorate for Health Protection region North
SWITZERLAND	Official Food Control Authority of the Canton of Zurich
Germany	Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit Bavarian Health and Food Safety Authority
Germany	Central Institute of the German Armed Forces Medical Service Koblenz - Department III Food ChemistryHessisches Landeslabor LHL Standort Wiesbaden

Annex 10

Procedure for the preparation of the spike of DIDP in sunflower oil and solvent (acetonitrile) for ILC

1. Washing of the mixing tank:

- The mixing tanks were washed in a washing machine, then filled with 6 L of deionised water (resistivity 18.0 MΩ.cm @ 25 °C) and mixed.
- The water was removed.
- The tank was filled with 2 L of ethanol (Fluka HPLC grade) and mixed for 2 hours.
- Ethanol was removed. The internal surface of the tank and the mixing device were rinsed with ethanol.
- The tank was filled with 2 L of n-hexane (Sigma-Aldrich HPLC grade) and mixed for 2 hours.
- n-hexane was removed. The internal surface of the tank and the mixing device were rinsed with n-hexane.
- The tanks and the mixing device were dried with a stream of pure nitrogen.

2. Washing of the glassware:

The glassware (100 ml amber glass crimp cap vials, 12 ml clear glass screw cap vials, 1 l flask class A and separatory funnel) was washed in a washing machine, then rinsed with deionised water (resistivity 18.0 MΩ.cm @ 25 °C) and pre-heated at 250°C for 2 h. Test was performed on a pre-washed and pre-heated vial with a portion of acetonitrile. Blank was confirmed as free of DIDP interference

3. Preparation of blank oil

- The empty mixing tank with its support was weighted (P1).
- 5 l of sunflower oil were transferred into the mixing tank and the mixing tank containing the oil was weighted (P2).
- The transferred blank sunflower oil was mixed for 2 h.
- The weight of the oil (P3) inside the mixing tank was obtained: P3= P2-P1
- The blank oil was distributed into 100 mL bottles (50 ml aliquots).

Note: all the weights were repeated 3 times and the average value was calculated.

4. Preparation of DIDP spiked oil

- The empty mixing tank with its support was weighted (P1)
- 5 l of sunflower oil were transferred into the mixing tank and the mixing tank containing the oil was weighted (P2)
- The established amount of DIDP was weighted on a glass support – see Table 1.
- The glass support was immersed into the oil using stainless steel rods.
- The transferred blank sunflower oil was mixed for 2 h

- The weight of the oil (P3) inside the mixing tank was obtained: P3= P2-P1.
- The spiked oil was distributed in 100 mL bottles (50 ml aliquots).

Note: all the weights were repeated 3 times and the average value was calculated.

	Target concentr.	Weighted amount,	Weighted oil,	Volume of oil,	Concentr. obtained
	mg/kg	**mg**	**kg**	**l**	
DIDP 1st level	3.25	14.82	4.501	4.907	3.29
DIDP 2nd level	9.15	40.21	4.501	4.907	8.93
DIDP 3rd level	13.4	60.06	4.498	4.907	13.35

5. Preparation of stock solution of DIDP in acetonitrile (approx. 10000 mg/l)

- The balance (balance AX 205) was checked against calibrated weight in the range 1000 mg + flask
- 997.51(64) mg of DIDP Jeyflax was dissolved in termostated to 20°C acetonitrile, the flask was filled up to the mark. The weight of empty and full flask was registered, giving a weight of 100 ml solution of DIDP in acetonitrile at 20°C P3=P2-P1= 78.03155 g;
- concentration of the resulting stock solution is 12.5015 mg/g

6. Preparation of DIDP in acetonitrile

- The balance (technical up to 6 kg) was checked by weighting 1000 ml deionized water at 20°C, obtaining a value of 996.4 g against 997.5 as it should be according to the t coefficient of expansion of water, and with 100 and 200 g calibrated weights.
- The empty 1 l class A volumetric flask was weighted (P4) with the stopper.
- An aliquot of 100 ml of preliminary termostated at 20°C acetonitrile was transferred into the 1 l class A volumetric flask.
- The calculated spiked volume (table 2) from stock solution of 12.5 mg/g was transferred with a 1000 ul syringe in a empty 1 ml preliminary weighted flask and the weigh registered (balance AX 205).
- The volume of DIDP solution in acetonitrile was transferred quantitatively into the 1 l flask, which was then filled up to the mark with termostated at 20°C acetonitrile.
- The flask filled with acetonitrile was weighted (P5) and the weight of acetonitrile calculated: P6= P5-P4.
- The spiked acetonitrile was distributed in 12 mL vials with screw caps via separatory funnel (10 ml aliquots)

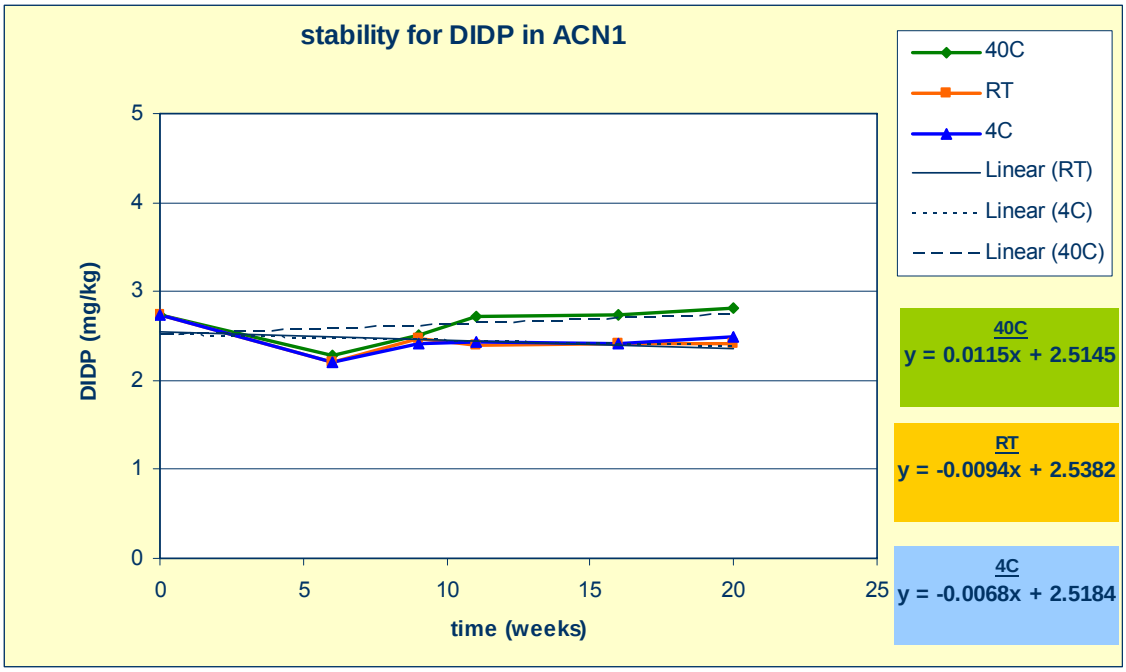
Note: all the weights were repeated 3 times and the average value was calculated.

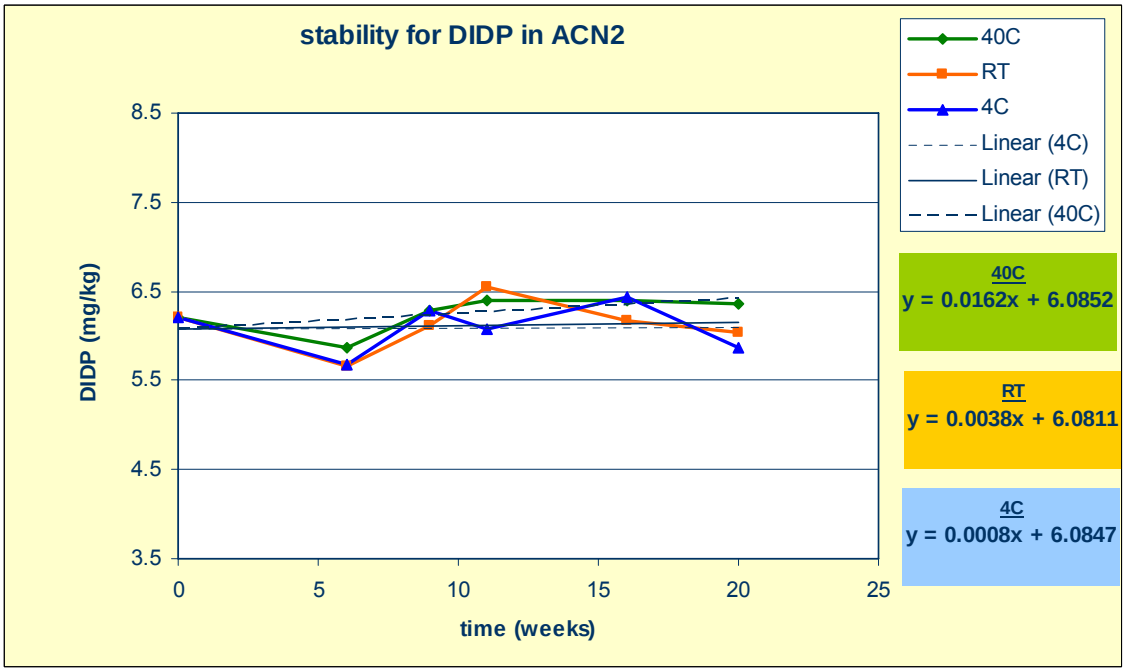
	weight of DIDP	weigth of stock solution 100 ml	Stock solution concentr.	spiked volume from 12.5 mg/g	Stock solution weigth.	spiked amount	Weighted acetonitrile	Obtained concentr	Uncer tainty
	mg	**g**	**mg/g**	**ul**	**mg**	**mg**	**kg**		
DIDP 1st level	975.51	78.03155	12.5015	200	157.30	1.97	0.7822	2.514	
DIDP 2nd level	975.51	78.03155	12.5015	500	396.16	4.95	0.7822	6.332	
DIDP 3rd level	975.51	78.03155	12.5015	750	588.60	7.36	0.7822	9.407	

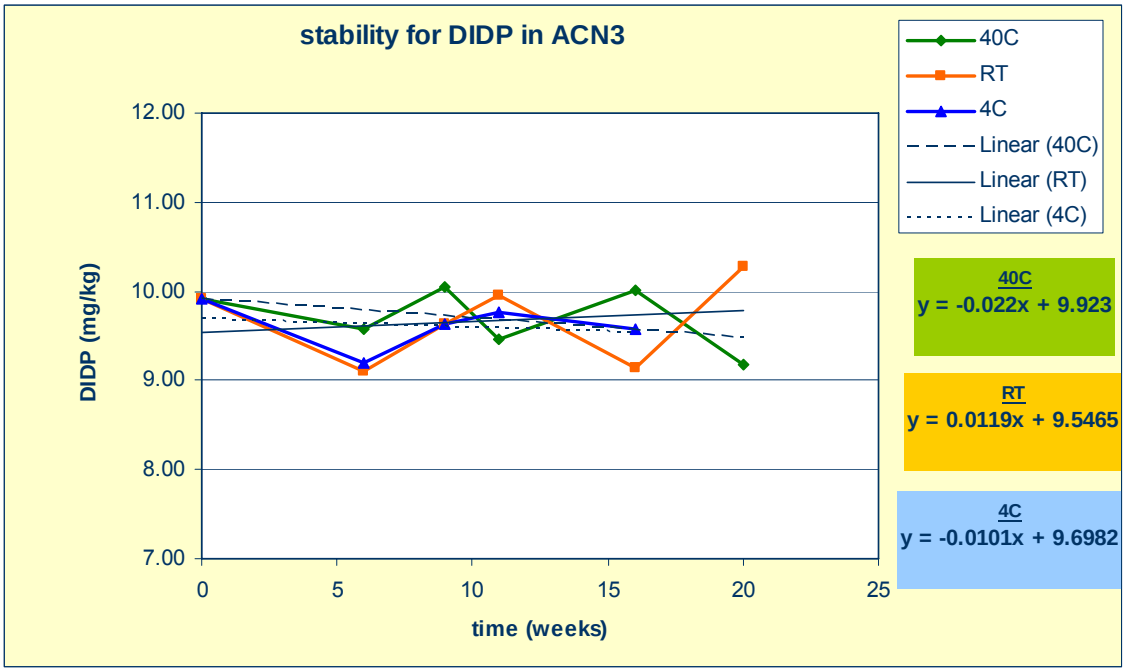
Annex 11: Results of the homogeneity study

Sample	Measurand	Unit	Mean	s(analytical) %	s(samples) %	Mode s(target)	s(target) %	ISO 13528 Check for sufficient homogeneity	Harmonized Protocol - test on significant heterogeneity
ACN1	DIDP	mg/kg	2.74	6.16	3.02	Horwitz	12.39	OK	OK
ACN2	DIDP	mg/kg	6.20	1.75	1.21	Horwitz	12.16	OK	OK
ACN3	DIDP	mg/kg	9.91	4.26	0.00	Horwitz	11.33	OK	OK
OIL1	DIDP	mg/kg	2.74	6.16	3.02	Horwitz	12.39	OK	OK
OIL2	DIDP	mg/kg	9.15	2.46	3.22	Horwitz	11.46	OK	OK
OIL3	DIDP	mg/kg	13.01	3.70	0.00	Horwitz	10.87	OK	OK

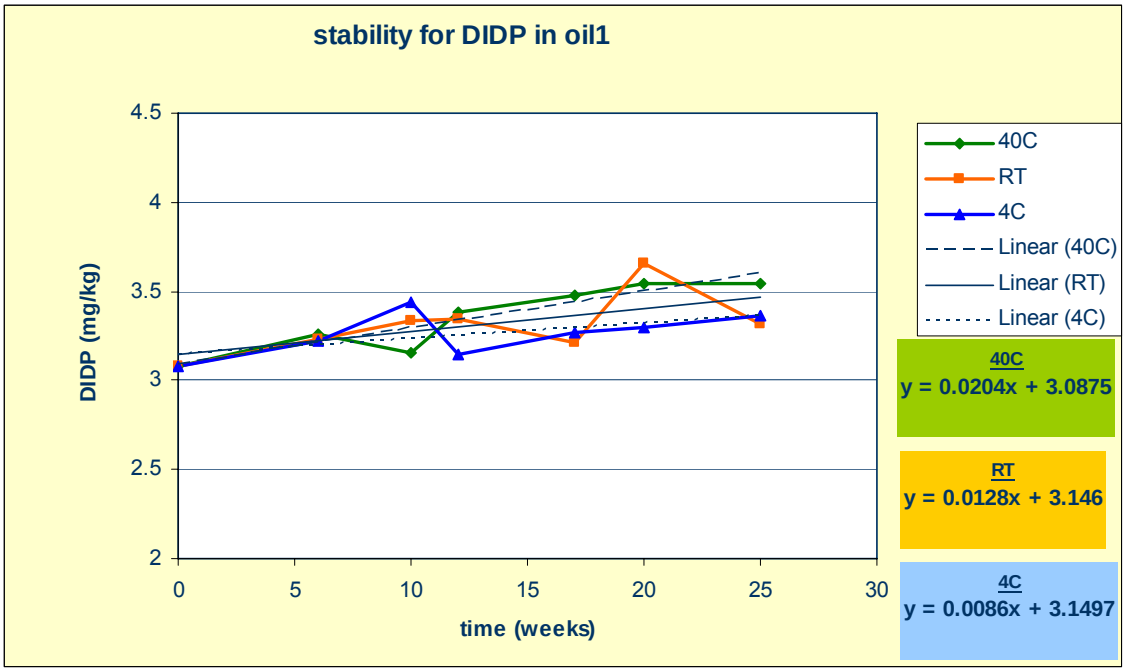
Annex 12: Results of the stability study

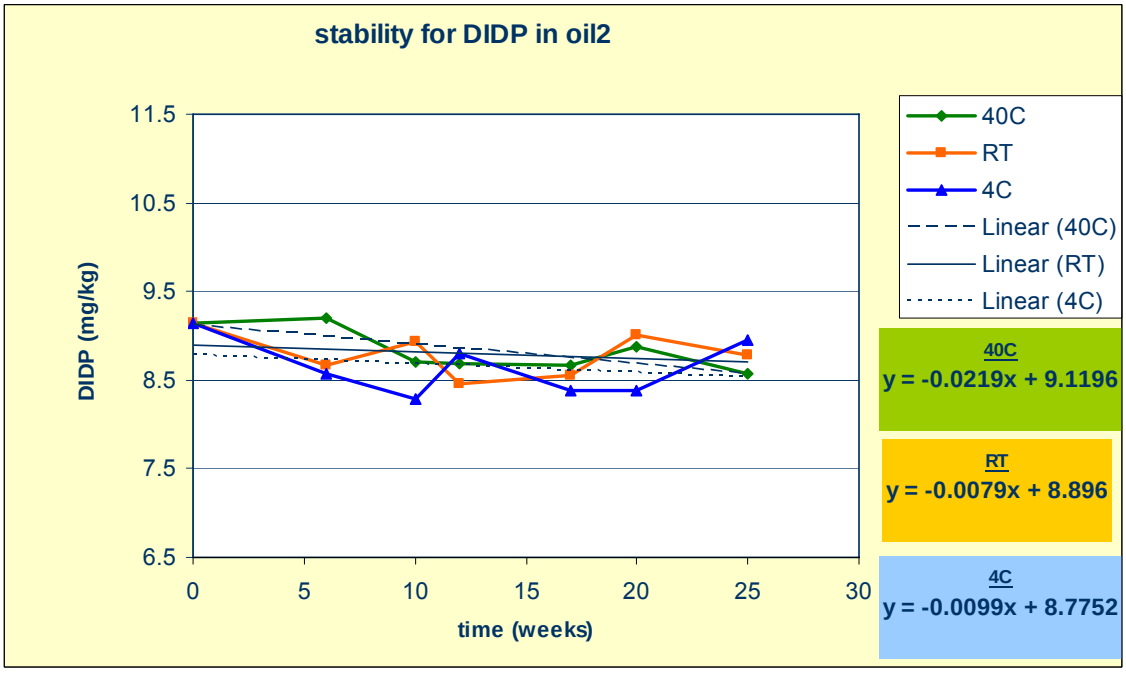


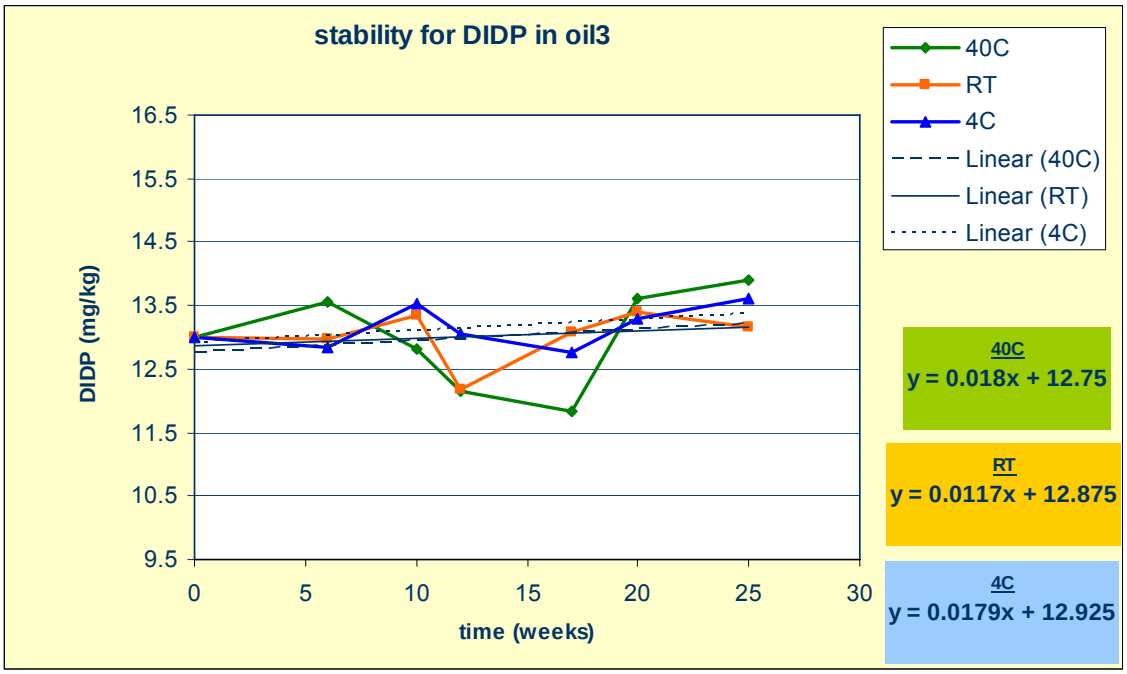




	test condition	reference value (mg/kg)	intercept (b0)	slope (b1)	s(b1)	t(α=0.95,g=4)·s(b1)
acn1	**40°C**	2.514	2.514	**0.0115**	0.0127	**0.0352**
	RT		2.538	**-0.0094**	0.0112	**0.0311**
	4°C		2.518	**-0.0068**	0.0117	**0.0324**
acn2	**40°C**	6.332	6.085	**0.0162**	0.0115	**0.0319**
	RT		6.081	**0.0038**	0.0201	**0.0558**
	4°C		6.085	**0.0008**	0.0195	**0.0542**
acn3	**40°C**	9.407	9.923	**-0.0220**	0.0220	**0.0609**
	RT		9.546	**0.0119**	0.0324	**0.0901**
	4°C		9.698	**-0.0101**	0.0253	**0.0807**


stability for DIDP in oil1
DIDP (mg/kg)
4.5
4
3.5
3
2.5
2
0
5
10
15
20
25
30
time (weeks)
40C
RT
4C
Linear (40C)
Linear (RT)
Linear (4C)
40C
y = 0.0204x + 3.0875
RT
y = 0.0128x + 3.146
4C
y = 0.0086x + 3.1497


stability for DIDP in oil2
DIDP (mg/kg)
11.5
10.5
9.5
8.5
7.5
6.5
0
5
10
15
20
25
30
time (weeks)
40C
RT
4C
Linear (40C)
Linear (RT)
Linear (4C)
40C
y = -0.0219x + 9.1196
RT
y = -0.0079x + 8.896
4C
y = -0.0099x + 8.7752



	test condition	reference value (mg/kg)	intercept (b0)	slope (b1)	s(b1)	t(α=0.95,g=5)·s(b1)
oil1	**40°C**	3.25	3.088	**0.0204**	0.0037	**0.0095**
	RT		3.146	**0.0128**	0.0074	**0.0191**
	4°C		3.150	**0.0086**	0.0052	**0.0133**
oil2	**40°C**	9.15	9.120	**-0.0219**	0.0087	**0.0223**
	RT		8.896	**-0.0079**	0.0127	**0.0326**
	4°C		8.775	**-0.0099**	0.0167	**0.0429**
oil3	**40°C**	13.4	12.750	**0.0180**	0.0399	**0.1025**
	RT		12.875	**0.0117**	0.0208	**0.0534**
	4°C		12.925	**0.0179**	0.0153	**0.0392**

European Commission
EUR 24387 – DG Joint Research Centre, Institute for Health and Consumer Protection
Report of the interlaboratory comparison DIDP in Oil, Stefanka Bratinova, Monica Multari, Stefano Spalla and Catherine Simoneau
Luxembourg: Publications Office of the European Union
2009 – 82 pp. – 21 x 29.7 cm
EUR - Scientific and Technical Research series; ISSN 1018-5593
ISBN 978-92-79-15867-4
doi:10.2788/97370

Abstract

The Institute for Health and Consumer Protection (IHCP) of the European Commission¿s Directorate-General Joint Research Centre hosts the European Union Reference Laboratory for Food Contact Materials (EURL-FCM). One of its core tasks is to organize interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs). This report presents the results of the second ILC of the EURL-FCM which focused on the determination of Di-isodecyl phthalate (DIDP) in an oil matrix. The aim was to develop and perform the validation of a method for the analysis of DIDP (as model substance for a technical mixture of phthalates) from oil (as simulant for fatty foods). This exercise was used both as proficiency testing and to validate a standard operating procedure (SOP) for the determination of DIDP in oil that was written by the EURL based on the most performant methods used by NRLs in the proficiency test of 2008. Participation of local laboratories under NRLs was encouraged (by producing 60 samples). There were 28 participants to whom samples were dispatched 24 of which submitted results. From the EURL-NRL network 23 laboratories out of 24 reported results. There were 2 guests from Germany that provided results as well. Participants were invited to report four replicates measurements under repeatability conditions. The ILC was closed permanently in the middle of October for statistical interpretation.
Based on the results in this precision experiment the method performance was assessed through evaluation of the repeatability and reproducibility standard deviation (SD) according to the mechanism described in ISO 5725 [11,12]. The assigned value and its uncertainty were obtained as a consensus values after applying the robust statistics to the results obtained from the participants. Laboratory results were rated with z and z¿ scores in accordance with ISO 13528 [1]. Standard deviations for proficiency assessment (also called target standard deviations) were set based on Horwitz equation. The participation of the laboratories was regarded as satisfactory for the aim of the precision experiment with regards of the numbers of received results thanks to the proactive involvement of the NRLs-FCM. As a conclusion for participation and laboratory performance, this ILC showed:
A noted increase in participation compared to the similar exercise of 2008. The number of laboratories submitting results for DIDP in oil rose from 17 to 25. This was due in part from the experience acquired in the previous year exercise as well as to the provision by the EURL of both the method description in a CEN like format as well as of the internal standard.
A great increase in laboratory performance compared to 2008 with 76-92% of successful achievement of results from the participants within the tolerance limits (range 76-92% depended on concentration level considered) compared to 59% in 2008. In particular the performance at the concentration level of the SML was 80% compared to 59% for the same exercise in 2008. The harmonisation of the procedure and following a harmonised method for determination of DIDP in oil in 2009 resulted in a decrease more then 2.5 times in the reproducibility SD from 37% to 14 % for the concentration level around SML of 9 mg/kg while the repeatability SD remained almost the same at 6.5%.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

JRC
EUROPEAN COMMISSION

LB-NA-24387-EN-C

ISBN 978-92-79-15867-4