Results of Proficiency Test
Preservatives in Body Lotion
(CMIT & MIT)
November 2019

Organised by: Institute for Interlaboratory Studies
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CONTENTS

1 INTRODUCTION

Methylisothiazolinone (MIT) and Methylchloroisothiazolinone (CMIT) are widely used as preservatives in liquid cosmetic and personal care products. Both chemicals inhibit bacterial growth in cosmetic products. CMIT and MIT could be allergenic and cytotoxic.
The mixture of CMIT and MIT as a preservative in rinse-off cosmetic products was authorized in cosmetics products through Annex V, entry 57, of Regulation (EC) No 1223/2009 ("Cosmetics Regulation") at a maximum concentration of 0.0015% (15 mg/kg) in a 3:1 mixture of CMIT : MIT since 16 July 2015.
No reference materials (RMs) for CMIT or MIT in cosmetics are available to optimise the determination of CMIT and MIT. As an alternative participation in a proficiency test may enable the laboratories to check their performance and thus to increase this comparability.

In 2018 on request of a number of laboratories, the Institute for Interlaboratory Studies (iis) has started a new proficiency test of the determination of CMIT (5-Chloro-2-methyl-4-isothiazolin-3-one) and MIT (2-Methyl-4-isothiazolin-3-one) in Body Lotion. It was decided to continue this proficiency test during the annual testing program 2019/2020.

In this interlaboratory study 14 laboratories in 11 different countries registered for participation. See appendix 2 for the number of participants per country.
In this report the results of the 2019 proficiency test are presented and discussed. This report is also electronically available through the iis website www.iisnl.com.

2 SET UP

The Institute for Interlaboratory Studies (iis) in Spijkenisse, the Netherlands, was the organizer of this proficiency test (PT). Sample analyses for fit-for-use and homogeneity testing were subcontracted to an ISO/IEC17025 accredited laboratory. It was decided to send one sample Body Lotion of 5 grams and labelled #19648, which was positive (artificially fortified) on CMIT (5-Chloro-2-methyl-4-isothiazolin-3-one) and MIT (2-Methyl-4-isothiazolin-3-one).
The participants were requested to report rounded and unrounded test results. The unrounded test results were preferably used for statistical evaluation.

2.1 QUALITY SYSTEM

The Institute for Interlaboratory Studies (iis) in Spijkenisse, the Netherlands, has implemented a quality system based on ISO/IEC17043:2010. This ensures strict adherence to protocols for sample preparation and statistical evaluation and 100% confidentiality of participant's data. Feedback from the participants on the reported data is encouraged and customer's satisfaction is measured on regular basis by sending out questionnaires.

2.2 PROTOCOL

The protocol followed in the organization of this proficiency test was the one as described for proficiency testing in the report 'iis Interlaboratory Studies: Protocol for the Organisation, Statistics and Evaluation' of June 2018 (iis-protocol, version 3.5). This protocol is electronically available through the iis website www.iisnl.com, from the FAQ page.

2.3 CONFIDENTIALITY STATEMENT

All data presented in this report must be regarded as confidential and for use by the participating companies only. Disclosure of the information in this report is only allowed by means of the entire report. Use of the contents of this report for third parties is only allowed by written permission of the Institute for Interlaboratory Studies. Disclosure of the identity of one or more of the participating companies will be done only after receipt of a written agreement of the companies involved.

2.4 SAMPLES

A regular body lotion was purchased from a local supermarket and was artificially fortified with a CMIT/MIT standard of approximately 30 mg/kg CMIT and 10 mg/kg MIT. After homogenization 24 cups of 15 ml were filled with approximately 5 grams of Body Lotion and labelled #19648.
The homogeneity of the subsamples #19648 was checked by determination of CMIT and MIT on five stratified randomly selected subsamples.

	CMIT in mg/kg	MIT in mg/kg
sample #19648-1	29.80	10.61
sample #19648-2	29.93	10.58
sample #19648-3	29.70	10.43
sample #19648-4	29.52	10.38
sample #19648-5	29.96	10.53

Table 1: homogeneity test results of subsamples #19648

From the above test results the repeatabilities were calculated and compared with 0.3 times the estimated reproducibility of the reference method in agreement with the procedure of ISO13528, Annex B2, in the next table.

	CMIT in mg/kg	MIT in mg/kg
r (observed)	0.50	0.27
reference method	Horwitz	Horwitz
0.3 * R (reference method)	2.40	0.35

Table 2: evaluation of the repeatabilities of subsamples #19648

The calculated repeatabilities of subsamples #19648 are in agreement with 0.3 times the reproducibility of the reference method. Therefore, homogeneity of the subsamples was assumed.

To each of the participating laboratories 1 sample labelled #19648 was sent on October 23, 2019.

2.5 ANALYSES

The participants were requested to determine the concentrations of CMIT (5-Chloro-2-methyl-4-isothiazolin-3-one) and MIT (2-Methyl-4-isothiazolin-3-one) applying the analytical procedure that is routinely used in the laboratory.

It was explicitly requested to treat the sample as if it was a routine sample and to report the test results using the indicated units on the report form and not to round the test results, but to report as much significant figures as possible. It was also requested not to report “less than” test results, which are above the detection limit, because such test results cannot be used for meaningful statistical evaluation.

To get comparable test results a detailed report form and a letter of instructions are prepared. On the report form the reporting units are given as well as the reference test methods (when applicable) that will be used during the evaluation. The detailed report form and the letter of instructions are both made available on the data entry portal www.kpmd.co.uk/sgs-iis-cts/. The participating laboratories are also requested to confirm the sample receipt on this data entry portal. The letter of instructions can also be downloaded from the iis website www.iisnl.com.

3 RESULTS

During five weeks after sample dispatch, the test results of the individual laboratories were gathered via the data entry portal www.kpmd.co.uk/sgs-iis-cts/. The reported test results are tabulated per determination in appendix 1 of this report. The laboratories are presented by their code numbers.

Directly after the deadline, a reminder was sent to those laboratories that had not reported test results at that moment. Shortly after the deadline, the available test results were screened for suspect data. A test result was called suspect in case the Huber Elimination Rule (a robust outlier test) found it to be an outlier. The laboratories that produced these suspect data were asked to check the reported test results (no reanalyzes). Additional or corrected test results are used for data analysis and original test results are placed under 'Remarks' in the test result tables in appendix 1. Test results that came in after the deadline were not taken into account in this screening for suspect data and thus these participants were not requested for checks.

3.1 STATISTICS

The protocol followed in the organization of this proficiency test was the one as described for proficiency testing in the report ‘iis Interlaboratory Studies: Protocol for the Organisation, Statistics and Evaluation’ of June 2018 (iis-protocol, version 3.5).

For the statistical evaluation, the *unrounded* (when available) figures were used instead of the rounded test results. Test results reported as ‘<…’ or ‘>…’ were not used in the statistical evaluation.

First, the normality of the distribution of the various data sets per determination was checked by means of the Lilliefors-test a variant of the Kolmogorov-Smirnov test and by the calculation of skewness and kurtosis. Evaluation of the three normality indicators in combination with the visual evaluation of the graphic Kernel density plot, lead to judgement of the normality being either 'unknown', 'OK', 'suspect' or 'not OK'. After removal of outliers, this check was repeated. If a dataset does not have a normal distribution, the (results of the) statistical evaluation should be used with due care.

According to ISO5725 the original test results per determination were submitted to Dixon's, Grubbs' and/or Rosner's outlier tests. Outliers are marked by D(0.01) for the Dixon's test, by G(0.01) or DG(0.01) for the Grubbs' test and by R(0.01) for the Rosner's test. Stragglers are marked by D(0.05) for the Dixon's test, by G(0.05) or DG(0.05) for the Grubbs' test and by R(0.05) for the Rosner's test. Both outliers and stragglers were not included in the calculations of averages and standard deviations.

For each assigned value, the uncertainty was determined in accordance with ISO13528. Subsequently the calculated uncertainty was evaluated against the respective requirement based on the target reproducibility in accordance with ISO13528. In this PT the criterion of ISO13528 paragraph 9.2.1 was met for all evaluated tests, therefore the uncertainty of all assigned values may be negligible and need not be included in the PT report.

Finally, the reproducibilities were calculated from the standard deviations by multiplying them with a factor of 2.8.

3.2 GRAPHICS

In order to visualize the data against the reproducibilities from literature, Gauss plots were made, using the sorted data for one determination (see appendix 1). On the Y-axis the reported test results are plotted. The corresponding laboratory numbers are on the X-axis.

The straight horizontal line presents the consensus value (a trimmed mean). The four striped lines, parallel to the consensus value line, are the +3s, +2s, -2s and -3s target reproducibility limits of the selected reference test method. Outliers and other data, which were excluded from the calculations, are represented as a cross. Accepted data are represented as a triangle.

Furthermore, Kernel Density Graphs were made. The Kernel Density Graph is a method for producing a smooth density approximation to a set of data that avoids some problems associated with histograms. Also, a normal Gauss curve was projected over the Kernel Density Graph for reference.

3.3 Z-SCORES

To evaluate the performance of the participating laboratories the z-scores were calculated. As it was decided to evaluate the performance of the participants in this proficiency test (PT) against the literature requirements, the z-scores were calculated using a target standard deviation. This results in an evaluation independent of the variation of this interlaboratory study.

The target standard deviation was calculated from the literature reproducibility by division with 2.8. In case no literature reproducibility was available, other target values were used. In some cases, a reproducibility based on former iis proficiency tests could be used.

When a laboratory did use a test method with a reproducibility that is significantly different from the reproducibility of the reference test method used in this report, it is strongly advised to recalculate the z-score, while using the reproducibility of the actual test method used, this in order to evaluate whether the reported test result is fit-for-use.

The z-scores were calculated according to:

$z_{(target)}$ = (test result - average of PT) / target standard deviation

The $z_{(target)}$ scores are listed in the test result tables in appendix 1.

Absolute values for z<2 are very common and absolute values for z>3 are very rare.
The usual interpretation of z-scores is as follows:

	$\|z\| < 1$	good
$1 <$	$\|z\| < 2$	satisfactory
$2 <$	$\|z\| < 3$	questionable
$3 <$	$\|z\|$	unsatisfactory

4 EVALUATION

During the execution of this proficiency test no problems were encountered with the dispatch of the samples. One participant did not report any test results. Finally, 13 laboratories reported 26 numerical test results. No outlying test results were observed. In proficiency studies outlier percentages of 3% - 7.5% are quite normal.

Both original data sets proved to have a normal Gaussian distribution.

4.1 EVALUATION PER COMPONENT

In this section, the test results are discussed per component.
The test methods, which were used by the various laboratories were taken into account for explaining the observed differences when possible and applicable. These test methods are also in the tables in appendix 1 together with the original data. The abbreviations used in these tables are explained in appendix 3.

Unfortunately, a suitable reference test method, providing the precision data, is not available for the determinations, therefore the calculated reproducibilities were compared against the reproducibility estimated from the Horwitz equation.

CMIT (CAS no. 26172-55-4): The determination of this component at a concentration level of 43 mg/kg may be problematic. No statistical outliers were observed. However, the calculated reproducibility is not in agreement with the calculated reproducibility estimated from the Horwitz equation.

MIT (CAS no. 2682-20-4): The determination of this component at a concentration level of 15 mg/kg may be problematic. No statistical outliers were observed. However, the calculated reproducibility is not in agreement with the calculated reproducibility estimated from the Horwitz equation.

4.2 PERFORMANCE EVALUATION FOR THE GROUP OF LABORATORIES

A comparison has been made between the estimated target reproducibilities and the reproducibilities as found for the group of participating laboratories. The number of significant test results, the average test result, the calculated reproducibility (2.8 * standard deviation) and the target reproducibility derived from the reference method (in casu Horwitz Equation) are compared in the next table.

Component	unit	n	average	2.8 * sd	R (target)
CMIT	mg/kg	13	43.2	23.9	11.0
MIT	mg/kg	13	15.0	7.9	4.5

Table 3: reproducibilities of tests on sample #19648

From the table above it can be concluded that without statistical calculations the group of participating laboratories do have some difficulties with the analysis of CMIT and MIT when compared with the target reproducibility. See also the discussion in paragraph 4.1.

4.3 COMPARISON OF THE PROFICIENCY TEST OF NOVEMBER 2019 WITH PREVIOUS PT

The uncertainties observed in the test results of the determination of CMIT and MIT in Body Lotion in the PT iis19H06 are listed in the next table.

Component	November 2019	November 2018	Target
CMIT	20%	10%	10%
MIT	19%	19%	12%

Table 4: comparison of uncertainties in iis proficiency tests.

It is remarkable that the variation of CMIT in the PT of 2019 is much higher that observed in 2019. Probably this is caused by the higher concentration of CMIT in this PT. However, the variation is comparable to the variations of MIT over both PT.

5 DISCUSSION

It is observed that all of the reporting laboratories would judge the sample in the same way and reject the sample for exceedingly too much CMIT/MIT present in accordance with the Annex V, entry 57, of Regulation (EC) No 1223/2009 ("Cosmetics Regulation") limit of 15 mg/kg.

In this proficiency test, the average of the homogeneity test results is not in line with the average (consensus value) from the PT results. There are several reasons for this. First the goal of homogeneity testing is very different from the goal of the evaluation of the reported PT results. In order to prove the homogeneity of the PT samples, a test method is selected with a high precision (smallest variation). The accuracy (trueness) of the test method is less relevant.

Secondly, the homogeneity testing is done by one laboratory only. The test results of this (ISO/IEC17025 accredited) laboratory will have a bias (systematic deviation) depending on the test method used. The desire to detect small variations between the PT samples leads to the use of a sensitive test method with high precision, which may be a test method with significant bias.

Also, each test result reported by the laboratories that participate in the PT will have a bias. However, some will have a positive bias and others a negative bias. These different biases compensate each other in the PT average (consensus value). Therefore, the PT consensus value may deviate form the average of the homogeneity test. At the same time the accuracy of the PT consensus value is more reliable than the accuracy of the average of the results of the homogeneity test.

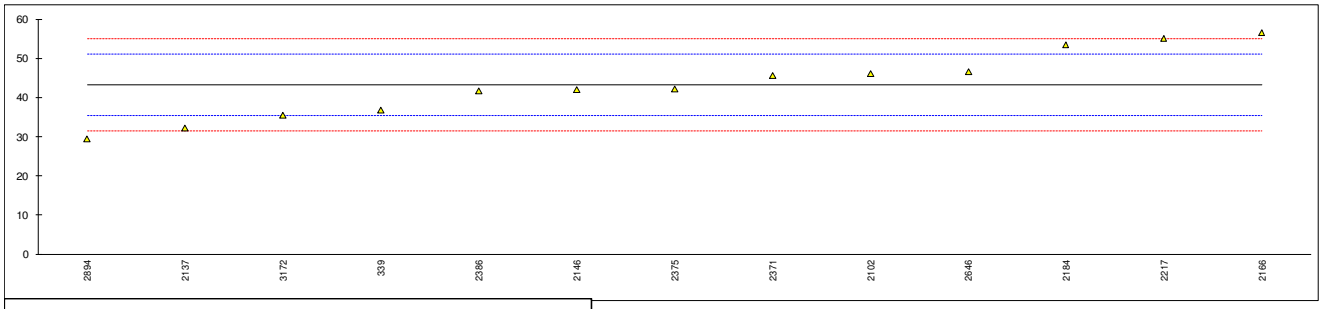
6 CONCLUSION

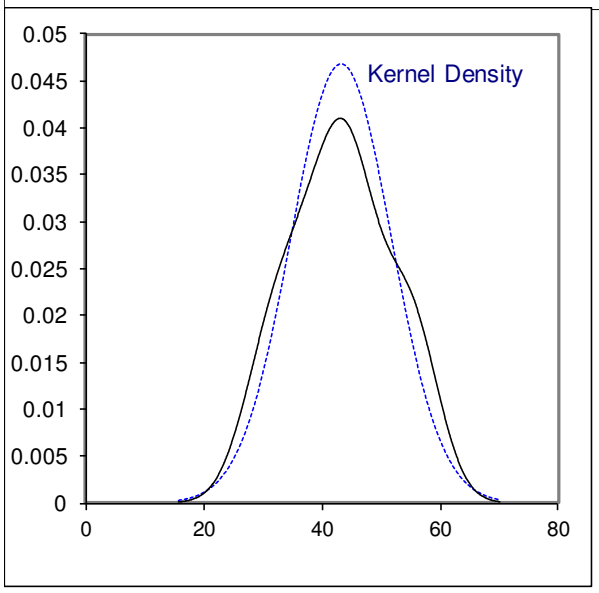
The observed variation in this interlaboratory study may not be caused by just one critical point in the analysis. Each participating laboratory will have to evaluate its performance in this study and decide about any corrective actions if necessary. Therefore, participation on a regular basis in this scheme could be helpful to improve the performance and the quality of the analytical results.

APPENDIX 1

Determination of CMIT (5-Chloro-2-methyl-4-isothiazolin-3-one) CAS no. 26172-55-4 in sample #19648; results in mg/kg

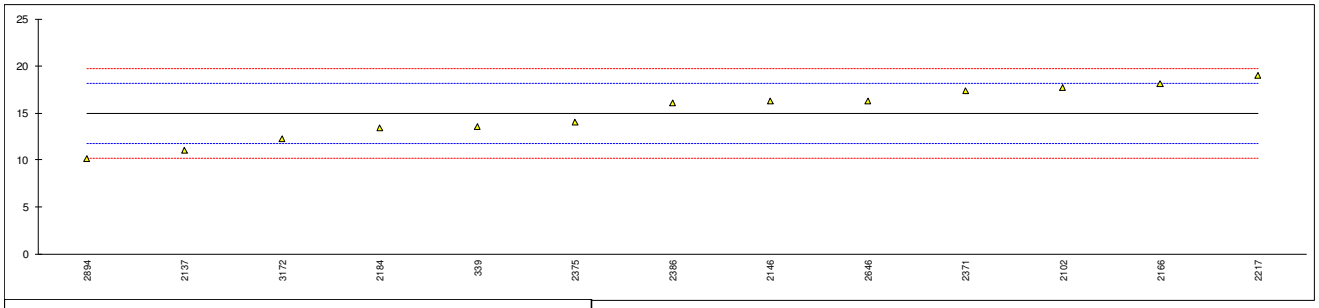
lab	method	value	mark	z(targ)	remarks
339		36.7		-1.66	
2102	In house	45.9		0.68	
2137	In house	32		-2.86	
2146	In house	41.967		-0.32	
2166	In house	56.49		3.38	
2184	EN71-10 & EN71-11	53.45		2.60	
2217	In house	54.982		3.00	
2371	In house	45.6		0.60	
2375	In house	42.14		-0.28	
2386	In house	41.5		-0.44	
2497		-----		-----	
2646	In house	46.555		0.85	
2894	In house	29.410		-3.52	
3172	In house	35.29	C	-2.02	First reported 12.22
	normality	OK			
	n	13			
	outliers	0			
	mean (n)	43.230			
	st.dev. (n)	8.5184	RSD = 20%		
	R(calc.)	23.851			
	st.dev.(Horwitz)	3.9237			
	R(Horwitz)	10.986			

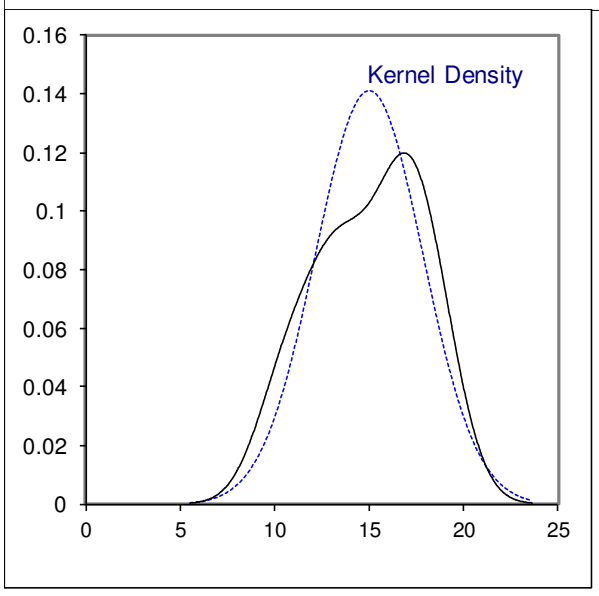




Determination of MIT (2-Methyl-4-isothiazolin-3-one) CAS no. 2682-20-4 in sample #19648; results in mg/kg

lab	method	value	mark	z(targ)	remarks
339		13.5		-0.93	
2102	In house	17.7		1.70	
2137	In house	11		-2.50	
2146	In house	16.235		0.79	
2166	In house	18.09		1.95	
2184	EN71-10 & EN71-11	13.36		-1.02	
2217	In house	19.018		2.53	
2371	In house	17.3		1.45	
2375	In house	13.97		-0.63	
2386	In house	16		0.64	
2497		-----		-----	
2646	In house	16.292		0.82	
2894	In house	10.087		-3.07	
3172	In house	12.22	C	-1.73	First reported 35.29
	normality	OK			
	n	13			
	outliers	0			
	mean (n)	14.982			
	st.dev. (n)	2.8333	RSD = 19%		
	R(calc.)	7.933			
	st.dev.(Horwitz)	1.5950			
	R(Horwitz)	4.466			





APPENDIX 2

Number of participants per country

1 lab in CYPRUS
1 lab in FINLAND
1 lab in FRANCE
3 labs in GERMANY
1 lab in HONG KONG
1 lab in HUNGARY
2 labs in ITALY
1 lab in SOUTH KOREA
1 lab in TAIWAN R.O.C.
1 lab in THE NETHERLANDS
1 lab in TURKEY

APPENDIX 3

Abbreviations

C = final test result after checking of first reported suspect test result
D(0.01) = outlier in Dixon's outlier test
D(0.05) = straggler in Dixon's outlier test
G(0.01) = outlier in Grubbs' outlier test
G(0.05) = straggler in Grubbs' outlier test
DG(0.01) = outlier in Double Grubbs' outlier test
DG(0.05) = straggler in Double Grubbs' outlier test
R(0.01) = outlier in Rosner's outlier test
R(0.05) = straggler in Rosner's outlier test
ex = test result excluded from statistical evaluation
n.a. = not applicable
n.e. = not evaluated
n.d. = not detected

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