Results of Proficiency Test
CMIT & MIT in Body Lotion
November 2018

Organised by: Institute for Interlaboratory Studies
Spijkenisse, the Netherlands

Author: ing. R.J. Starink
Corrector: ing. A.S. Noordman-de Neef

Report: iis18H04

January 2019

CONTENTS

1 INTRODUCTION

Methylisothiazolinone (MIT) and Methylchloroisothiazolinone (CMIT) are widely used as preservatives found 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.

On request of a number of laboratories, the Institute for Interlaboratory Studies (iis) decided to set up 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 during the annual testing program 2018/2019.

In this interlaboratory study 7 laboratories from 6 different countries registered for participation. See appendix 2 for the number of participants per country. In this report the results of the 2018 proficiency test are presented and discussed. This report is also electronically available through the iis website ww.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/IEC 17025 accredited laboratory. It was decided to send in this proficiency test one sample of Body Lotion (labelled #18710) 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 in Spijkenisse, the Netherlands, has implemented a quality system based on IEC/ISO17043:2010. 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 organisation of this proficiency test was the one as described for proficiency testing in the report 'iis Interlaboratory Studies: Protocol for the Organization, 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 supermarket and was artificially fortified with a CMIT/MIT standard to a theoretically concentration of 17.0 mg/kg CMIT and 5.6 mg/kg MIT. From this batch 16 cups of 15 ml were filled with approximately 10 grams Body Lotion and labelled #18710. The homogeneity of the subsamples #18710 was checked by determination of CMIT and MIT on five stratified randomly selected subsamples. See the following table for the test results.

	CMIT in mg/kg	*MIT in mg/kg*
sample #18710-1	18.02	6.88
sample #18710-2	17.04	6.52
sample #18710-3	17.39	6.84
sample #18710-4	17.68	6.92
sample #18710-5	17.35	6.93

Table 1: homogeneity test results of subsamples #18710

From the above test results the repeatability was calculated and compared with 0.3 times the reproducibility of the reference test method in agreement with the procedure of ISO13528, Annex B2, in the next table:

	CMIT in mg/kg	*MIT in mg/kg*
r (observed)	1.04	0.48
reference method	Horwitz	Horwitz
0.3 * R (ref. method)	1.53	0.69

Table 2: evaluation of the repeatability of subsamples #18710

The calculated repeatability of samples #18710 is in agreement with 0.3 times the reproducibility of the reference method. Therefore, homogeneity of the subsamples #18710 was assumed.

To each of the participating laboratories 1 sample labelled #18710 was sent on October 24, 2018.

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 results, but report as much significant figures as possible. It was also requested not to report ‘less than’ results, which are above the detection limit, because such results cannot be used for meaningful statistical evaluations.

To get comparable 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 appropriate reference test method 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 and 2 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 reanalyses). 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 ISO 5725 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 as met for all evaluated tests, therefore, the uncertainty of all assigned values maybe 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. This 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 occurred.
One participant did not report any test results. The 6 participants reported 12 numerical test results. Observed were 2 outlying test results, which is 17% of the numerical test results. In proficiency studies, outlier percentages of 3% - 7.5% are quite normal.

4.1 EVALUATION PER COMPONENT

In this section, the results are discussed per component. All statistical results reported on the textile samples are summarised in appendix 1.

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 (CASno. 26172-55-4): The determination of this component at a concentration level of 21.8 mg/kg was not problematic. One statistical outlier was observed. However, the calculated reproducibility after rejection of the statistical outlier is in full agreement with the calculated reproducibility estimated from the Horwitz equation.

MIT (CASno. 2682-20-4): The determination of this component at a concentration level of 6.6 mg/kg may be problematic. One statistical outlier was observed. The calculated reproducibility after rejection of the statistical outlier 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 reproducibilities as declared by the relevant reference method and the reproducibilities as found for the group of participating laboratories. The number of significant test results, the average result, the calculated reproducibility (2.8*sd) and the target reproducibility derived from the reference method (in casu Horwitz Equation) are presented in the next table.

Component	unit	n	average	2.8 * sd	R (target)
CMIT	mg/kg	5	21.8	6.3	6.1
MIT	mg/kg	5	6.6	3.4	2.2

Table 3: reproducibilities of tests on sample #18710

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 MIT when compared with the target reproducibility, but not with the analysis of CMIT. See also the discussions in paragraphs 4.1.

4.3 UNCERTAINTIES OF THE PROFICIENCY TEST OF NOVEMBER 2018

The uncertainties observedy in the test results of the determination of CMIT and MIT in Body Lotion in the PT: iis18H04 are listed in the next table:

Component	November 2018	Target (Horwitz)
CMIT	10%	10%
MIT	19%	12%

Table 4: development of relative uncertainties (RSD).

5 CONCLUSION

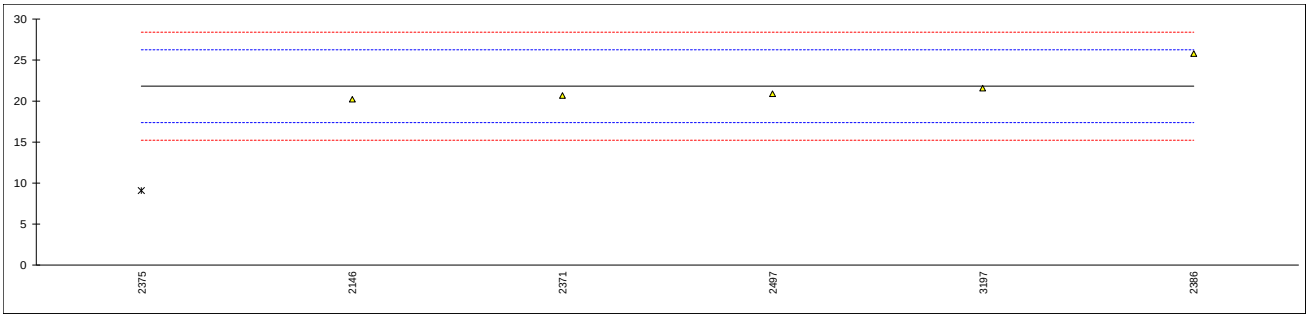
It is observed that one of the six reporting laboratories would judge the sample different and would accept the sample for not exceeding too much CMIT/MIT present in accordance with the Annex V, entry 57, of Regulation (EC) No 1223/2009 ("Cosmetics Regulation") limit of 15 mg/kg. The other five particiapnts would reject the sample.

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

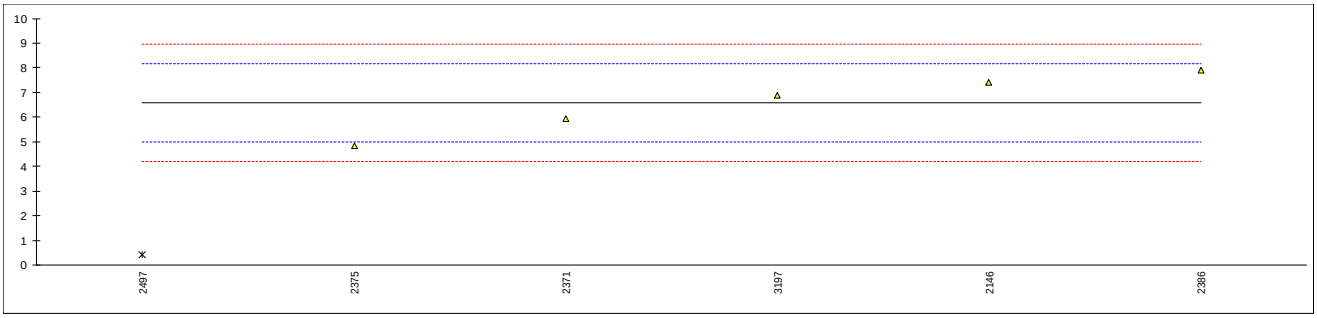
Determination of CMIT (5-Chloro-2-methyl-4-isothiazolin-3-one) CAS no. 26172-55-4 in sample #18710; results in mg/kg

lab	method	value	mark	z(targ)	remarks
2146	In house	20.201		-0.73	
2371	In house	20.6		-0.54	
2375	In house	9.12	G(0.05)	-5.78	
2386	In house	25.7		1.78	
2493		-----		-----	
2497	In house	20.858		-0.43	
3197	In house	21.6		-0.09	
	normality	Not OK			
	n	5			
	outliers	1			
	mean (n)	21.792	RSD% =10.3%		
	st.dev. (n)	2.2435			
	R(calc.)	6.282			
	st.dev.(Horwitz)	2.1927			
	R(Horwitz)	6.140			



Determination of MIT (2-Methyl-4-isothiazolin-3-one) CAS no. 2682-20-4 in sample #18710; results in mg/kg

lab	method	value	mark	z(targ)	remarks
2146	In house	7.400		1.01	
2371	In house	5.93		-0.84	
2375	In house	4.84	C	-2.21	First reported 3.21
2386	In house	7.91		1.65	
2493		-----		-----	
2497	In house	0.41	C,G(0.05)	-7.79	First reported 0.807
3197	In house	6.9		0.38	
	normality	OK			
	n	5			
	outliers	1			
	mean (n)	6.596	RSD% = 18.6%		
	st.dev. (n)	1.2240			
	R(calc.)	3.427			
	st.dev.(Horwitz)	0.7945			
	R(Horwitz)	2.225			



APPENDIX 2

Number of participants per country

1 lab in FINLAND
1 lab in GERMANY
1 labs in HUNGARY
1 labs in ITALY
1 lab in TAIWAN R.O.C.
2 labs 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

Literature:

1 iis-Interlaboratory Studies: Protocol for the Organisation, Statistics and Evaluation, June 2018

2 P.L. Davies, Fr Z. Anal. Chem, 351, 513, (1988)

3 W.J. Conover, Practical; Nonparametric Statistics, J. Wiley&Sons, NY, p.302, (1971)

4 ISO 5725, (1986)

5 ISO 5725, parts 1-6, (1994)

6 ISO 13528:05

7 M. Thompson and R. Wood, J. AOAC Int, 76, 926, (1993)

8 W.J. Youden and E.H. Steiner, Statistical Manual of the AOAC, (1975)

9 G. Rohm, J. Bohnen & H. Kruessmann, GIT Labor-Fachzeitschrift, p 1080, 11, (1997)

10 Bernard Rosner, Percentage Points for a Generalized ESD Many-Outlier Procedure, T*echnometrics*, 25(2), 165-172, (1983)

11 Analytical Methods Committee Technical brief, No 4, January 2001

12 P.J. Lowthian and M. Thompson, The Royal Society of Chemistry, Analyst, 127, 1359-1364, (2002)

13 Horwitz, W and Albert, R, J. AOAC Int, 79, 3, 589, (1996)

14 EU Public Consultation on Methylisothiazone (MIT) Ban for Leave-on Cosmetic Products Launched, May11, 2016 original article from CIRS