Results of Proficiency Test Preservatives in Body Milk February 2019

Organised by: Institute for Interlaboratory Studies
Spijkenisse, the Netherlands

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CONTENTS

1 INTRODUCTION

Parabens are widely used as preservatives found in liquid cosmetic and personal care products to provide bacterial growth in cosmetic products.
Parabens are regulated in cosmetic products through Annex V, entry 57, of Regulation (EC) No 1223/2009 ("Cosmetics Regulation") at a maximum concentration of 0.4% for single ester and 0.8% for mixtures of esters since 16 July 2015.
No reference materials (RMs) for individual Parabens in cosmetics are available to optimise the determination of Parabens. 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 Methylparaben, Ethylparaben, Propylparaben, Isobutylparaben, Butylparabenand Phenoxyethanol in Body Milk during the annual testing program 2018/2019.
In this interlaboratory study 13 laboratories from 10 different countries registered for participation. See appendix 3 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 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 Milk (labelled #19507) which was artificially fortified on Methylparaben, Ethylparaben, Propylparaben, Isobutylparaben, Butylparaben and Phenoxyethanol.
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 ISO/IEC 17043:2010. 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 Milk was purchased from a supermarket and artificially fortified with the preservatives: Methylparaben, Ethylparaben, Propylparaben, Isobutylparaben, Butylparaben and Phenoxyethanol.
From this batch 35 cups of 15 ml were filled with approximately 5 grams Body Milk and labelled #19507.
The homogeneity of the subsamples #19507 was checked by determination of Methylparaben and Phenoxyethanol by using an inhouse test method on five stratified randomly selected subsamples. See the following table for the test results.

	Methylparaben in %M/M	Phenoxyethanol in %M/M
sample #19507-1	0.0490	0.2714
sample #19507-2	0.0486	0.2681
sample #19507-3	0.0493	0.2707
sample #19507-4	0.0489	0.2701
sample #19507-5	0.0498	0.2714

Table 1: homogeneity test results of subsamples #19507

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

	Methylparaben in %M/M	Phenoxyethanol in %M/M
r (observed)	0.0014	0.0038
reference method	Horwitz	Horwitz
0.3 * R (ref. method)	0.0026	0.0111

Table 2: evaluation of the repeatabilities of subsamples #19507

The calculated repeatabilities of sample #19507 is in agreement with 0.3 times the reproducibility of the reference method. Therefore, homogeneity of the subsamples #19507 was assumed.

To each of the participating laboratories 1 sample labelled #19507 was sent on January 16, 2019.

2.5 ANALYSES

The participants were requested to determine the concentrations of Methylparaben, Ethylparaben, Propylparaben, Isobutylparaben, Butylparaben and Phenoxyethanol applying the analytical procedure that is routinely used in the laboratory.
It was also requeted to report if the laboratory was accredited for the requested components that were determined and what sample amount was used for intake.

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.

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 with the dispatchof the samples. All participants reported test results. The 13 participants reported 67 numerical test results. Observed were 3 outlying test results, which is 4.5% of the numerical test results. In proficiency studies, outlier percentages of 3% - 7.5% are quite normal.

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

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. The abbreviations, used in these tables, are listed in appendix 4.

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.

Methylparaben: This determination may be problematic. No statistical outliers were observed. However, the calculated reproducibility is not in agreement with the estimated reproducibility using the Horwitz equation.

Ethylparaben: This determination may be problematic. No statistical outliers were observed. However, the calculated reproducibility is not in agreement with the estimated reproducibility using the Horwitz equation.

Propylparaben: This determination may be problematic. One statistical outlier was observed. The calculated reproducibility after rejection of the statistical outlier is not in agreement with the estimated reproducibility using the Horwitz equation.

Isobutylparaben: This determination mat be problematic. No statistical outliers were observed. However, the calculated reproducibility is not in agreement with the estimated reproducibility using the Horwitz equation.

Butylparaben: This determination was not problematic. One statistical outlier was observed. However, the calculated reproducibility after rejection of the statistical outlier is in agreement with the estimated reproducibility using the Horwitz equation.

Phenoxyethanol: This determination may be problematic. One statistical outlier was observed. The calculated reproducibility after rejection of the statistical outlier is not in agreement with the estimated reproducibility using 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)
Methylparaben	%M/M	11	0.0681	0.0254	0.0114
Ethylparaben	%M/M	11	0.0199	0.0059	0.0040
Propylparaben	%M/M	11	0.0084	0.0028	0.0019
Isobutylparaben	%M/M	10	0.0090	0.0035	0.0020
Butylparaben	%M/M	10	0.0156	0.0031	0.0033
Phenoxyethanol	%M/M	11	0.4010	0.1316	0.0515

Table 3: reproducibilities of tests on sample #19507

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 parabens when compared with the strict target reproducibility estimated from the Horwitz equation. See also the discussions in paragraphs 4.1.

4.3 UNCERTAINTIES OF THE PROFICIENCY TEST OF JANUARY 2019

The uncertainties observedy in the test results of the determination of Parabens in Body Milk in the PT: iis19H02 are listed in the next table:

Component	January 2019	Target (Horwitz)
Methylparaben	13%	6%
Ethylparaben	11%	7%
Propylparaben	12%	8%
Isobutylparaben	14%	8%
Butylparaben	7%	7%
Phenoxyethanol	12%	5%

Table 4: development of relative uncertainties (RSD).

4.4 EVALUATION ANALYTICAL DETAILS

For this PT, some analytical details were requested, see appendix 2. Based on the answers given by the participants the following can be summarized:
Eight of the thirteen reporting participants (= 62%) mentioned that they are accredited for determination of preservatives in Cosmetics.
The second question was about the intake of the sample used for the determination. Seven of the thirteen reporting laboratories used 0.25 or 0.5 gram, three other participants used respectively 1, 3 or 5 gram for sample intake.

5 CONCLUSION

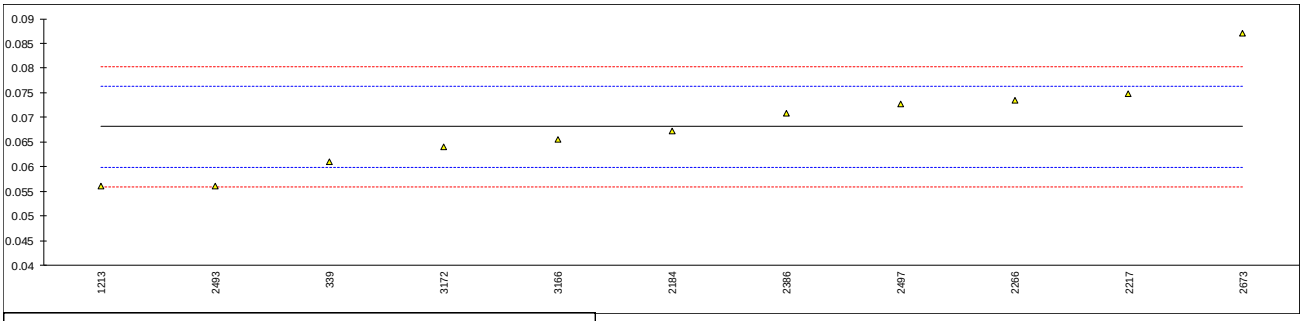
It is observed that none of the reporting laboratories would judge the sample different and would accept the sample for not exceeding too much Parabens present in accordance with the Annex V, entry 57, of Regulation (EC) No 1223/2009 ("Cosmetics Regulation") limit of 0.4 %M/M (single ester) or 0.8%M/M (mixture of esters).

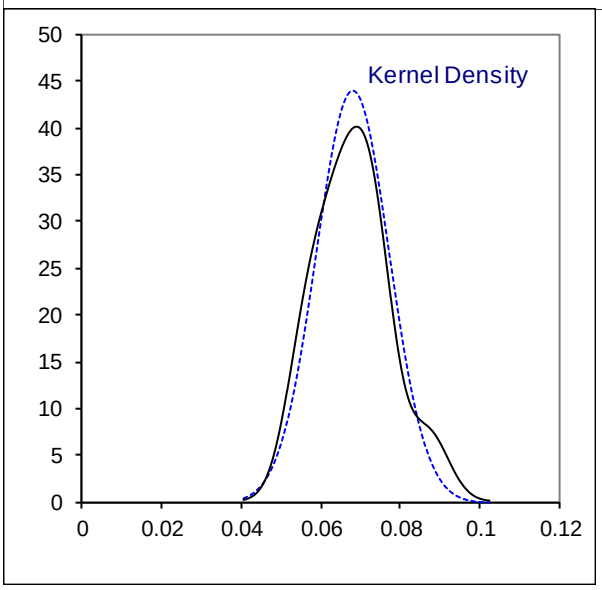
The observed variation in this interlaboratory study may not be caused by just one critical point in the analysis. Each participating laboratory will have to evaluate its performance in this study and decide about any corrective actions if necessary. Therefore, participation on a regular basis in this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

APPENDIX 1

Determination of Methylparaben CAS No. 99-76-3 in sample #19507; results in %M/M

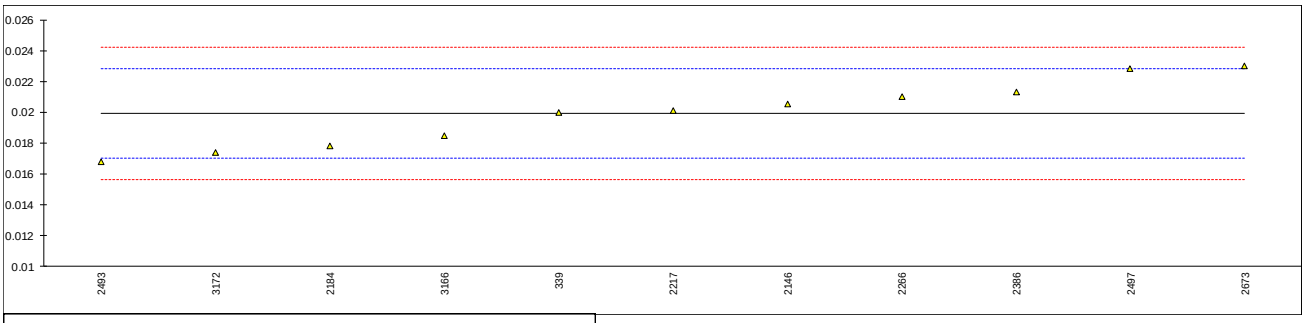
lab	method	value	mark	z(targ)	remarks
339	In house	0.061		-1.74	
1213		0.056		-2.96	
2146		-----		-----	
2184	In house	0.0673		-0.19	
2217	In house	0.074879		1.66	
2266	In house	0.073408		1.30	
2386	In house	0.0709		0.69	
2493		0.0561		-2.94	
2497	In house	0.0728		1.15	
2673	In house	0.087	C	4.63	First reported 0.245
3166	In house	0.0656		-0.61	
3172	In house	0.064		-1.00	
3197	In house	<0,05		<-4.43	Possibly a false negative test result?
	normality	OK			
	n	11			
	outliers	0			
	mean (n)	0.06809			
	st.dev. (n)	0.009087	RSD	= 13%	
	R(calc.)	0.02544			
	st.dev.(Horwitz)	0.004081			
	R(Horwitz)	0.01143			

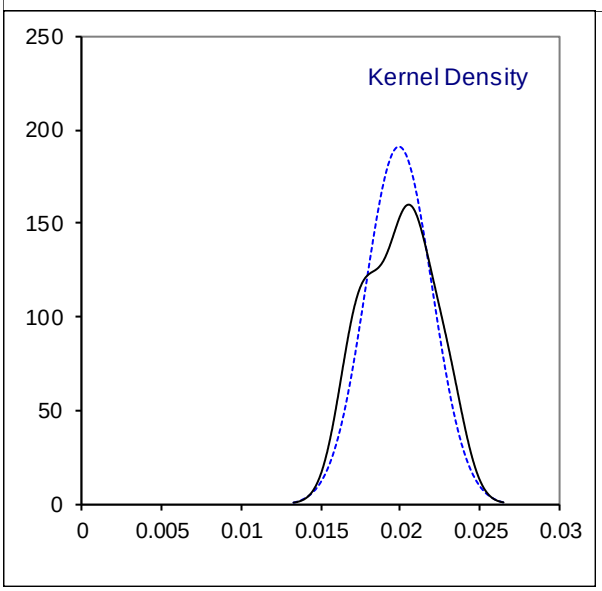




Determination of Ethylparaben CAS No. 120-47-8 in sample #19507; results in %M/M

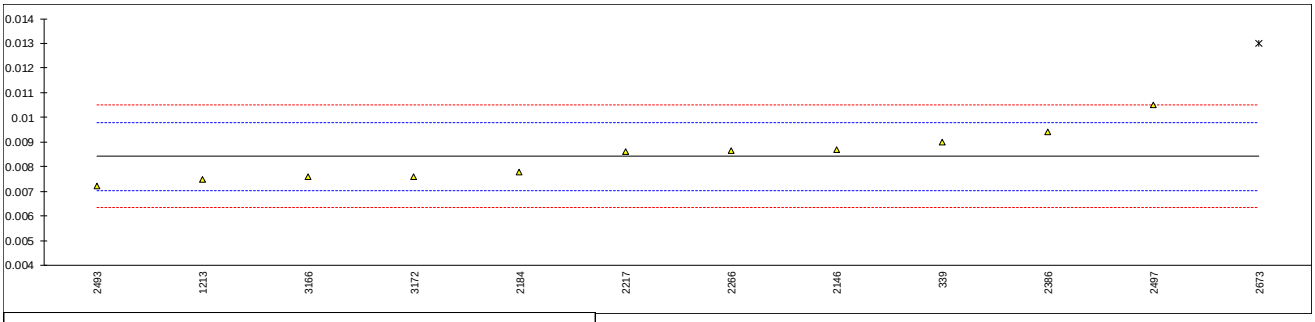
lab	method	value	mark	z(targ)	remarks
339	In house	0.020		0.05	
1213		-----		-----	
2146	In house	0.0205		0.40	
2184	In house	0.0178		-1.48	
2217	In house	0.020121		0.13	
2266	In house	0.020984		0.74	
2386	In house	0.0213		0.95	
2493		0.0168		-2.18	
2497	In house	0.0228		2.00	
2673	In house	0.023		2.14	
3166	In house	0.0185		-0.99	
3172	In house	0.0174		-1.76	
3197	In house	<0,05		-----	
	normality	OK			
	n	11			
	outliers	0			
	mean (n)	0.01993			
	st.dev. (n)	0.002093	RSD	= 11%	
	R(calc.)	0.00586			
	st.dev.(Horwitz)	0.001437			
	R(Horwitz)	0.00402			

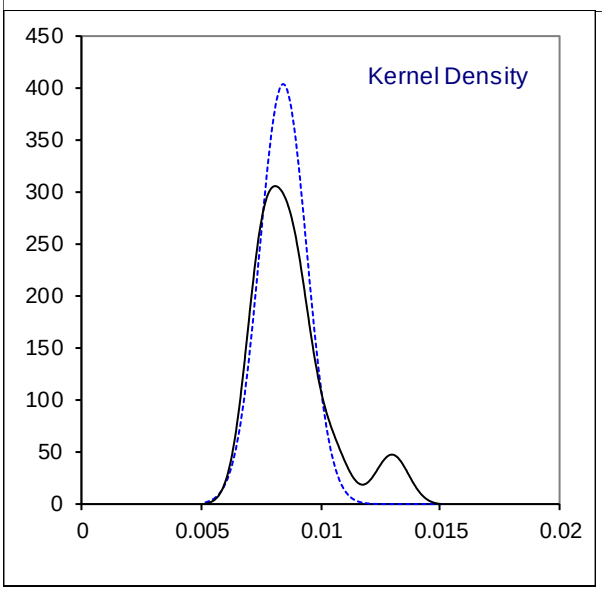




Determination of Propylparaben CAS No. 94-13-3 in sample #19507; results in %M/M

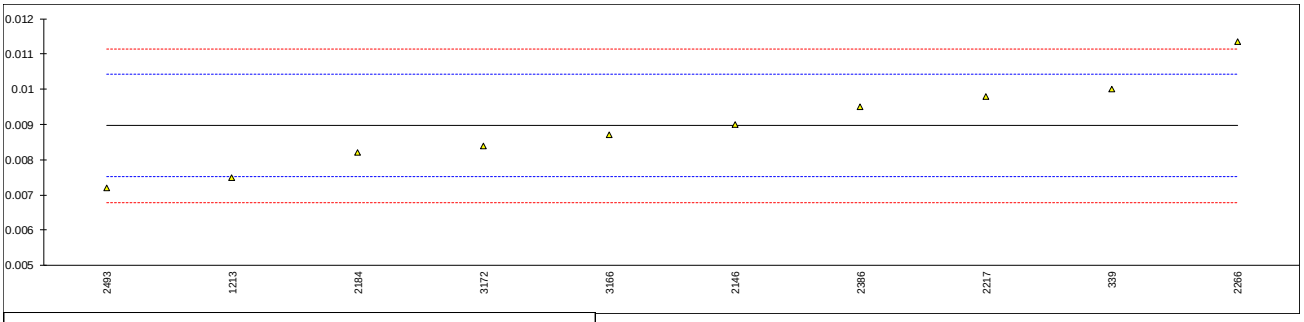
lab	method	value	mark	z(targ)	remarks
339	In house	0.009		0.84	
1213		0.0075		-1.33	
2146	In house	0.0087		0.41	
2184	In house	0.0078		-0.90	
2217	In house	0.008610		0.28	
2266	In house	0.008669		0.36	
2386	In house	0.0094		1.42	
2493		0.00723		-1.72	
2497	In house	0.0105		3.01	
2673	In house	0.013	C,G(0.05)	6.63	First reported 0.017
3166	In house	0.0076		-1.18	
3172	In house	0.0076		-1.18	
3197	In house	<0,05		-----	
	normality	OK			
	n	11			
	outliers	1			
	mean (n)	0.00842			
	st.dev. (n)	0.000990	RSD	= 12%	
	R(calc.)	0.00277			
	st.dev.(Horwitz)	0.000691			
	R(Horwitz)	0.00194			

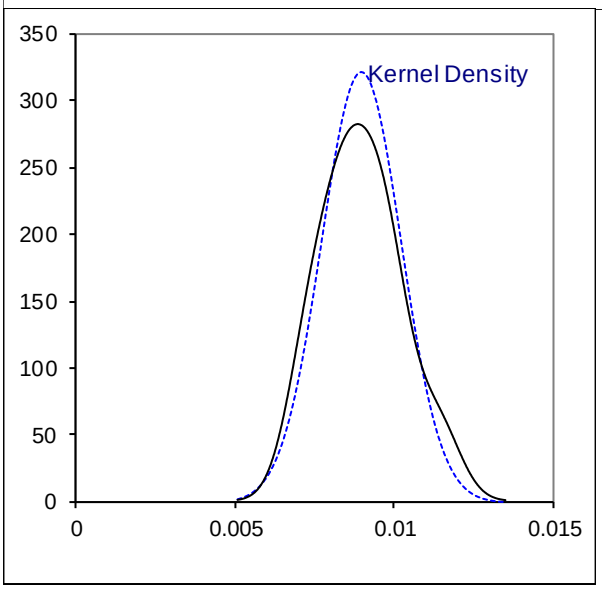




Determination of Isobutylparaben CAS No. 4247-02-3 in sample #19507; results in %M/M

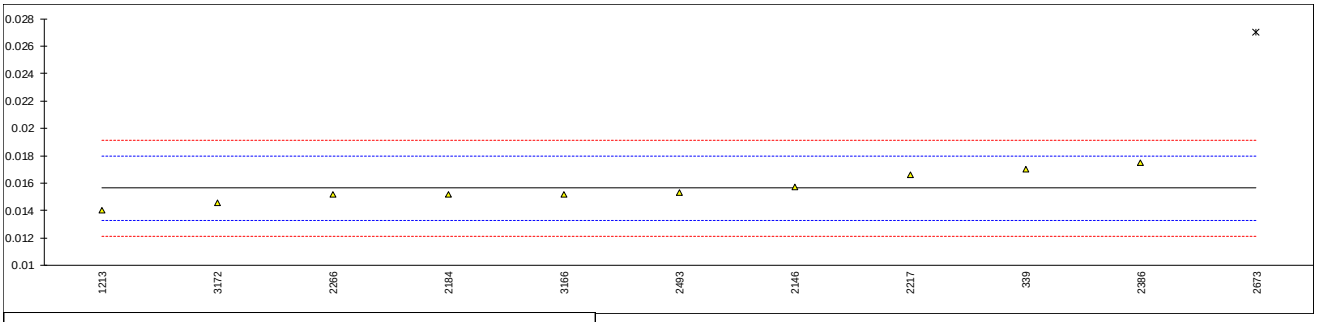
lab	method	value	mark	z(targ)	remarks
339	In house	0.010		1.42	
1213		0.0075		-2.01	
2146	In house	0.0090		0.05	
2184	In house	0.0082		-1.05	
2217	In house	0.009785		1.13	
2266	In house	0.011352		3.27	
2386	In house	0.0095		0.73	
2493		0.00721		-2.41	
2497		-----	W	-----	Reported 0.0328 (= sum of Butyl and Isobutyl paraben)
2673		-----		-----	
3166	In house	0.0087		-0.36	
3172	In house	0.0084		-0.77	
3197	In house	<0,05		-----	
	normality	OK			
	n	10			
	outliers	0			
	mean (n)	0.00896			
	st.dev. (n)	0.001244	RSD	= 14%	
	R(calc.)	0.00348			
	st.dev.(Horwitz)	0.000729			
	R(Horwitz)	0.00204			

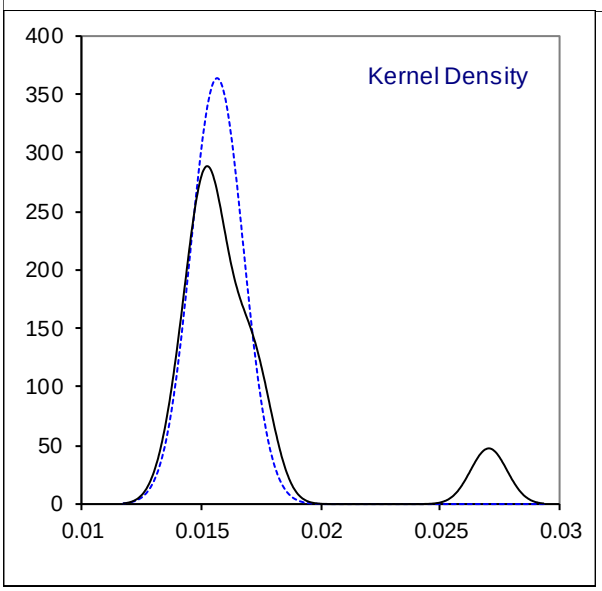




Determination of Butylparaben CAS No. 94-26-8 in sample #19507; results in %M/M

lab	method	value	mark	z(targ)	remarks
339	In house	0.017		1.17	
1213		0.014		-1.39	
2146	In house	0.0157		0.06	
2184	In house	0.0152		-0.37	
2217	In house	0.016631		0.86	
2266	In house	0.015164		-0.40	
2386	In house	0.0175		1.60	
2493		0.0153		-0.28	
2497		-----	W	-----	Reported 0.0328 (= sum of Butyl and Isobutyl paraben)
2673	In house	0.027	C,G(0.01)	9.73	First reported 0.045
3166	In house	0.0152		-0.37	
3172	In house	0.0146		-0.88	
3197	In house	<0,05		-----	
	normality	OK			
	n	10			
	outliers	1			
	mean (n)	0.01563			
	st.dev. (n)	0.001095	RSD	= 7%	
	R(calc.)	0.00307			
	st.dev.(Horwitz)	0.001169			
	R(Horwitz)	0.00327			

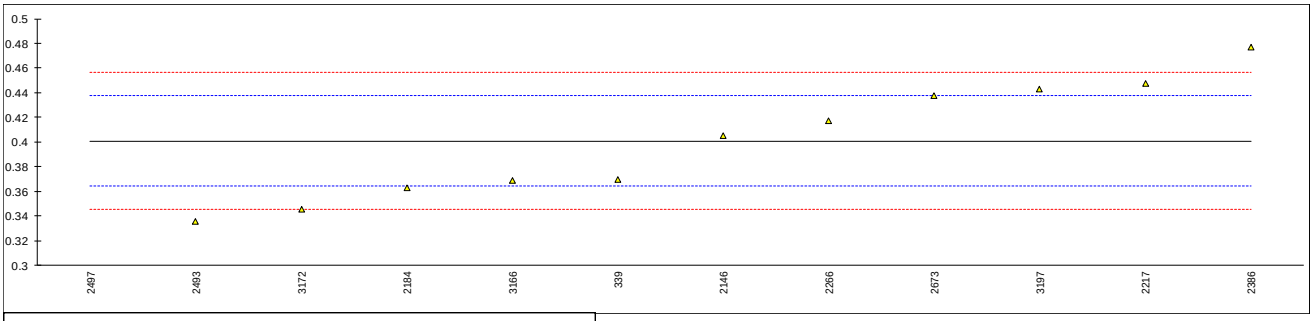


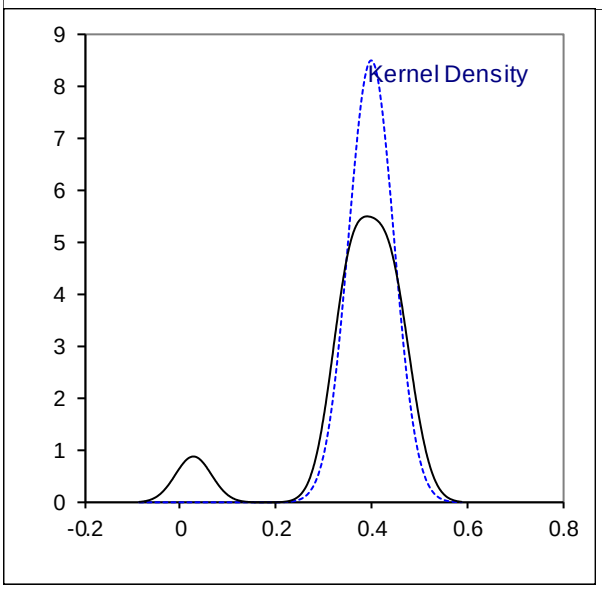


Determination of Phenoxyethanol CAS No. 122-99-6 in sample #19507; results in %M/M

lab	method	value	mark	z(targ)	remarks
339	In house	0.370		-1.68	
1213		-----		-----	
2146	In house	0.405		0.22	
2184	In house	0.3631		-2.06	
2217	In house	0.447654		2.53	
2266	In house	0.417218		0.88	
2386	In house	0.4769		4.12	
2493		0.336		-3.53	
2497	In house	0.0295	G(0.01)	-20.18	
2673	In house	0.438		2.01	
3166	In house	0.3689		-1.74	
3172	In house	0.3453		-3.03	
3197	In house	0.443		2.28	

normality	OK		
n	11		
outliers	1		
mean (n)	0.40101		
st.dev. (n)	0.047016	RSD	= 12%
R(calc.)	0.13164		
st.dev.(Horwitz)	0.018405		
R(Horwitz)	0.05153		





APPENDIX 2

Analytical details

lab	ISO17025 accredited	Intake in gram
339	No	5
1213	Yes	--
2146	Yes	1
2184	No	0.5
2217	No	0.5
2266	Yes	0.25
2386	Yes	3
2493	No	0.5
2497	---	--
2673	Yes	0.5
3166	Yes	0.5
3172	Yes	--
3197	Yes	0.5

APPENDIX 3

Number of participants per country

1 lab in FINLAND
2 labs in FRANCE
1 lab in GERMANY
1 lab in HONG KONG
2 labs in HUNGARY
2 labs in ITALY
1 lab in SERBIA
1 lab in TURKEY
1 lab in UNITED STATES OF AMERICA
1 lab in VIETNAM

APPENDIX 4

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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