**Institute for
Interlaboratory Studies**

Results of Proficiency Test
Aniline in Textile
May 2023

CONTENTS

Appendices:

1   INTRODUCTION

Aniline is a chemical compound that is used as raw material for the synthesis of modern synthetic dyes. Firmly bound in the crystal structure of the synthetic dye it is not suspected to be harmful. However, free Aniline is classified as carcinogenic and mutagenic. In 2021 the European Union issued Commision Directive EU 2021/903 with limit values for Aniline in textile for the use in toys. Next to this, both OEKOTEX® and Bluesign® mention limits for the presence of Aniline in textile.

On request of a number of participants the Institute for Interlaboratory Studies (iis) decided to organize a proficiency scheme for the determination of Aniline in Textile.
In this interlaboratory study 24 laboratories in 12 countries registered for participation, see appendix 3 for the number of participants per country. In this report the results of the Aniline in Textile 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 analyzes for fit-for-use and homogeneity testing were subcontracted to an ISO/IEC17025 accredited laboratory.
It was decided to send one textile sample of approximately 3 grams labelled #23600.
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/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 a 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 batch of black cotton which was made positive on Aniline by a third party was selected. The batch was cut into small pieces. After homogenization 40 small plastic bags were filled with approximately 3 grams each and labelled #23600.
The homogeneity of the subsamples was checked by determination of Aniline in accordance with ISO14362 on 8 stratified randomly selected subsamples.

	Aniline in mg/kg
sample #23600-1	224.75
sample #23600-2	224.28
sample #23600-3	234.47
sample #23600-4	247.64
sample #23600-5	239.56
sample #23600-6	230.71
sample #23600-7	240.77
sample #23600-8	229.28

Table 1: homogeneity test results of subsamples #23600

From the above test results the repeatability was calculated and compared with 0.3 times the reproducibility obtained from eleven iis PTs of EN14362 test data from 2010 – 2021 (see iis memo 2202, lit. 13) in agreement with the procedure of ISO13528, Annex B2 in the next table.

	Aniline in mg/kg
r (observed)	23.09
reference method	iis memo 2202
0.3 x R (reference method)	41.27

Table 2: evaluation of the repeatability of subsamples #23600

The calculated repeatability is in agreement with 0.3 times the reproducibility obtained from previous iis PTs of EN14362 test data. Therefore, homogeneity of the subsamples was assumed.

To each of the participating laboratories one textile sample labelled #23600 was sent on May 3, 2023.

2.5   ANALYZES

The participants were requested to determine the Aniline content.
To ensure homogeneity it was requested not to use less than 0.5 gram per determination. It was also requested to report if the laboratory was accredited for the determined component and to report some analytical details.

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 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 evaluations.

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 method (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 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.

The assigned value is determined by consensus based on the test results of the group of participants after rejection of the statistical outliers and/or suspect data.

According to ISO13528 all (original received or corrected) results per determination were submitted to outlier tests. In the iis procedure for proficiency tests, outliers are detected prior to calculation of the mean, standard deviation and reproducibility. For small data sets, Dixon (up to 20 test results) or Grubbs (up to 40 test results) outlier tests can be used. For larger data sets (above 20 test results) Rosner's outlier test can be used. 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. 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 (dotted line) was projected over the Kernel Density Graph (smooth line) for reference. The Gauss curve is calculated from the consensus value and the corresponding standard deviation.

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 (derived from e.g. ISO or ASTM test methods), 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, like Horwitz or an estimated reproducibility based on former iis proficiency tests.

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. Therefore, 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

In this interlaboratory study some problems were encountered with the dispatch of the samples. One participant reported test results after the final reporting date and two other participants did not report a test result. In total 22 laboratories reported 21 numerical test results. Observed was 1 outlying test result, which is 4.8%. In proficiency studies outlier percentages of 3% - 7.5% are quite normal.

The data set proved to have a normal Gaussian distribution.

4.1 EVALUATION PER COMPONENT

In this section the reported 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. The test methods are also in the table together with the original data in appendix 1. The abbreviations, used in this table, are explained in appendix 4.

For the determination of Aniline in Textile the same test method is used as for AZO Dyes in Textile, which is ISO14362. Unfortunately, precision data are mentioned in this test method only for a few aromatic amines. Furthermore, the precision data mentioned in ISO14382 is often not for a large concentration range or not conclusive. As alternative for the aromatic amines not mentioned in the test method iis could have used an estimated target reproducibility calculated with the Horwitz equation. Unfortunately, this would have given quite a strict target value for the reproducibility.

Therefore, iis decided to use the iis PT data gathered from 2010 to 2021 in the PT for AZO Dyes in Textile to estimate a more realistic target reproducibility for the evaluation of the quality of the test results. Furthermore, it was decided to use the same target reproducibly for all aromatic amines and thus also for Aniline. The average relative standard deviation over all iis PTs and components for Textile is 21%. This investigation is summarized in iis memo 2202.

Aniline: This determination was not problematic. One statistical outlier was observed. The calculated reproducibility after rejection of the statistical outlier is in agreement with the target reproducibility as derived from iis memo 2202.

4.2 PERFORMANCE EVALUATION FOR THE GROUP OF LABORATORIES

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

Component	unit	n	average	2.8 * sd	R(target)
Aniline	mg/kg	20	210	128	123

Table 3: reproducibility on sample #23600

Without further statistical calculations it can be concluded that for Aniline there is a good compliance of the group of participating laboratories with the reference method.

4.3 OVERVIEW OF THE PROFICIENCY TEST OF MAY 2023

	May 2023
Number of reporting laboratories	22
Number of test results	21
Number of statistical outliers	1
Percentage of statistical outliers	4.8%

Table 4: overview of this proficiency test

In proficiency tests outlier percentages of 3% - 7.5% are quite normal.

The performance of the determination of the proficiency test, expressed as relative standard deviation (RSD) of the PT, is shown in the next table.

	May 2023	iis memo 2202
Aniline	22%	21%

Table 5: development of the uncertainties over the years

4.4   EVALUATION OF THE ANALYTICAL DETAILS

For this PT some analytical details were requested which are listed in appendix 2. Based on the answers given by the participants the following can be summarized:
-   Sixteen of the participants mentioned that they are accredited for the determination of Aniline in Textile.
-   Nine of the participants used the samples as received and thirteen participants further cut the samples prior to analysis.
-   Ten of the participants used 0.5 grams and eight participants used 1 gram as sample intake.
-   Five of the participants used mechanical shaking as extraction technique, three participants used thermal desorption and three participants used a water bath.
-   The majority of the participants (thirteen) used MTBE as extraction solvent. The other participants reported a wide variety of other extraction solvents, e.g. a citrate buffer, Ethyl acetate or Xylene.
-   Seven participants used an extraction time of 30 minutes and seven used 60 minutes. The others used between 15 and 90 minutes.
-   Fifteen participants used an extraction temperature of 70 °C. The other two reporting laboratories used <50 °C and 200 °C.
-   Thirteen participants followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column, seven followed ISO14362-1 Annex E and did not use a diatomaceous earth column and one participant applied direct reduction with a diatomaceous earth column.

As the calculated reproducibility is in line with target reprocucibilty and as the group of participants is small, no further statistical analysis has been performed to the analytical details.

5   DISCUSSION

All of the reporting participants, except one, were able to detect Aniline in sample #23600.

The test results of this interlaboratory study were compared to the Ecolabelling Standards and Requirements for Textiles in EU (see table below). It was noticed that not all of the participants would have made identical decisions about the acceptability of the textile sample for the presence of Aniline. All reporting participants, except one, would have rejected sample #23600 for all categories.

Ecolabel	textile toy material	baby clothes	in direct skin contact	no direct skin contact
EU 2021/903	30 mg/kg	--	--	--
OEKO-TEX® 100	--	20 mg/kg	50 mg/kg	50 mg/kg
Bluesign® RSL	--	30 mg/kg	30 mg/kg	30 mg/kg

Table 6: Ecolabelling Standards and Requirements for Textiles in EU

6   CONCLUSION

Although it can be concluded that the majority of the participants has no problem with the determination of Aniline in the textile sample of this PT, 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 thus increase of the quality of the analytical results.

APPENDIX 1

Determination of Aniline on sample #23600; results in mg/kg

lab	method	value	mark	z(targ)	remarks
339	In house	not detected	f-?	-----	possible false negative test result?
551		-----		-----	
840	ISO14362-1	200		-0.22	
2131	In house	173		-0.83	
2250	ISO14362-1	236		0.60	
2265	ISO14362-1	289.0		1.80	
2326	ISO14362-1	234.06		0.56	
2370	ISO14362-1	208.91		-0.02	
2375	ISO14362-1	236.68		0.62	
2386	ISO14362-1	203.2		-0.15	
2459	EN14362-1	205.6		-0.09	
2485	ISO14362-1	298.29		2.01	
2561		-----		-----	
2590	ISO14362-1	197.62		-0.27	
2638	ISO14362-1	156.38		-1.21	
2643	ISO14362-1	210.62		0.02	
2644	ISO14362-1	233.0		0.53	
2743	ISO14362-1	187.40		-0.50	
2858	ISO14362-1	114.04		-2.17	
2977	ISO14362-1	163.78		-1.04	
3176	ISO14362-1	62.72	C,R(0.05)	-3.34	first reported: 43.27
3210	ISO14362-1	254.67		1.02	
3222	ISO14362-1	240.7		0.71	
6191	ISO14362-1	149.127		-1.37	

normality	OK	
n	20	
outliers	1	
mean (n)	209.604	
st.dev. (n)	45.6486	RSD = 22%
R(calc.)	127.816	
st.dev.(iis memo 2202)	44.0168	
R(iis memo 2202)	123.247	
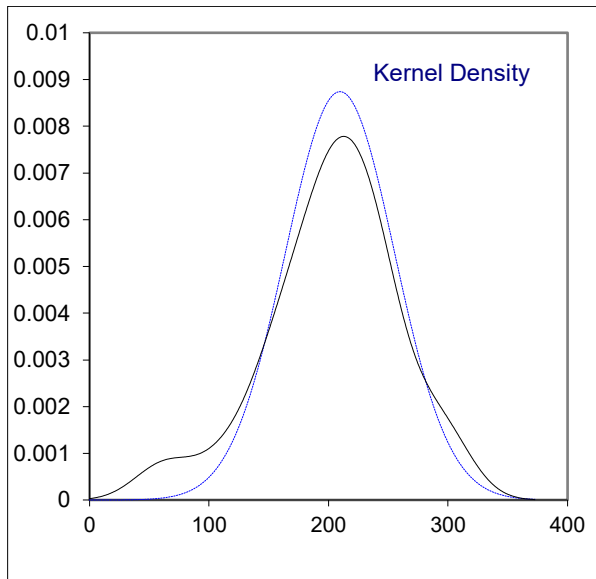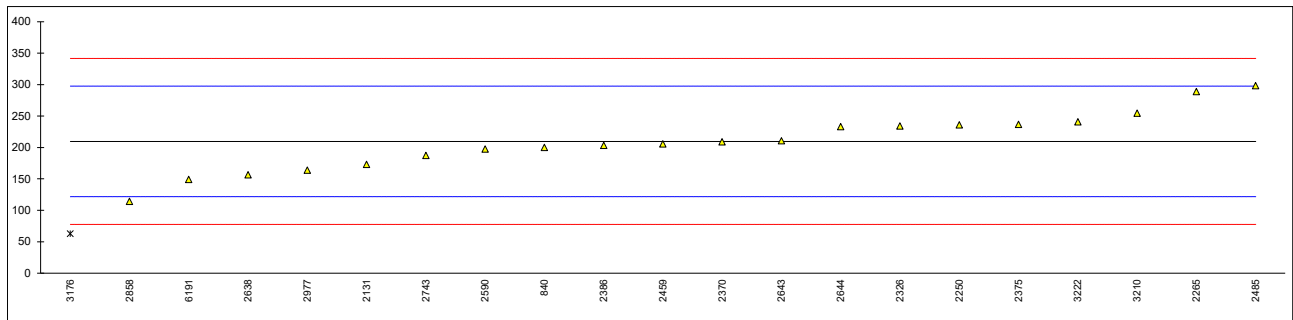
APPENDIX 2 Analytical details

lab	ISO/IEC 17025 accr.	Sample preparation	Sample intake used (grams)	Extraction technique
339	No	Used as received	0.36	Reflux extraction without sample contact
551	---	---		---
840	Yes	Further cut	0.5	Thermal Desorption
2131	Yes	Used as received	0.5	Mechanical Shaking
2250	Yes	Further cut	0,5	Water bath
2265	Yes	Used as received	0,5	Ultrasonic
2326	Yes	Further cut	1	Mechanical Shaking
2370	Yes	Further cut	0.5	Mechanical Shaking
2375	Yes	Further cut	1	---
2386	Yes	Further cut	0.5	Thermal Desorption
2459	Yes	Used as received	1	Mechanical Shaking
2485	Yes	Further cut	0.5	Mechanical Shaking
2561	---	---		---
2590	Yes	Further cut	1	---
2638	No	Further cut	0.7	Diatomaceous earth column
2643	Yes	Used as received	0.5	---
2644	Yes	Used as received	0.5	Ultrasonic
2743	Yes	Used as received		---
2858	Yes	Further cut	0.602	---
2977	No	Used as received	1	Thermal Desorption
3176	Yes	Used as received	1	Water bath
3210	---	Further cut	1	Heating device
3222	No	Further cut	0.5	Thermal water bath
6191	No	Further cut	1,0084	---

lab	Extraction solvent	Extraction time in min.	Extraction temp. in °C	Absorption procedure
339	Xylene	40	200	I followed ISO14362-1 Annex E and did NOT use the diatomaceous earth column
551	---	---	---	---
840	BUFFER	60	70	I followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column
2131	MTBE	40 + 30 min	70C	I followed ISO14362-1 Annex E and did NOT use the diatomaceous earth column
2250	MTBE	30 minutes	70°C	I followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column
2265	TBME	60	70	---
2326	Ethylacetate	15 minutes	N/A	I followed ISO14362-1 Annex E and did NOT use the diatomaceous earth column
2370	ACN/MTBE	60 minutes	70 °C	I followed ISO14362-1 Annex E and did NOT use the diatomaceous earth column
2375	-	-	-	I followed ISO14362-1 Annex E and did NOT use the diatomaceous earth column
2386	Citrate buffer	2 x 30 min	70°C	I followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column
2459	TBME	30 minutes	70°C	I followed ISO14362-1 Annex E and did NOT use the diatomaceous earth column
2485	Citrate buffer / sodium dithionite	30 + 30 min	70°C	I followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column
2561	---	---	---	---
2590	mtbe	---	---	I followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column
2638	TBME	15-20 mints	<50 C	I followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column
2643				I followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column
2644	MTBE	30 min	70°C	I followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column
2743	MTBE	60	70	I followed ISO14362-1 Annex E and did NOT use the diatomaceous earth column
2858	TBME and methanol	90	70	I followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column
2977	TMBE	60'	70°C	I followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column
3176	citrate buffer / sodium dithionite	60	70	I followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column
3210	MTBE / ACN	2*30 min	70°C	I followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column
3222	TBME / ACN	60 + 60 min	70°C / RT	I followed ISO14362-1 chapter 10.4 and used the diatomaceous earth column
6191	Ethylacetate	---	---	Direct reduction, no treatment. The lab used the diatomaceous earth column.

APPENDIX 3

Number of participants per country

    1 lab in BANGLADESH
    1 lab in BRAZIL
   3 labs in FRANCE
   3 labs in GERMANY
   5 labs in ITALY
    1 lab in KOREA, Republic of
   3 labs in PAKISTAN
   2 labs in SWITZERLAND
    1 lab in TAIWAN
   2 labs in TURKEY
    1 lab in UNITED KINGDOM
    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
E	= calculation difference between reported test result and result calculated by iis
W	= test result withdrawn on request of participant
ex	= test result excluded from statistical evaluation
n.a.	= not applicable
n.e.	= not evaluated
n.d.	= not detected
fr.	= first reported
f+?	= possibly a false positive test result?
f-?	= possibly a false negative test result?

Literature

1   iis Interlaboratory Studies, Protocol for the Organisation, Statistics & Evaluation, June 2018
2   ISO5725:86
3   ISO5725 parts 1-6:94
4   ISO13528:05
5   M. Thompson and R. Wood, J. AOAC Int, 76, 926, (1993)
6   W.J. Youden and E.H. Steiner, Statistical Manual of the AOAC, (1975)
7   P.L. Davies, Fr. Z. Anal. Chem, 331, 513, (1988)
8   J.N. Miller, Analyst, 118, 455, (1993)
9   Analytical Methods Committee, Technical Brief, No 4, January 2001
10   P.J. Lowthian and M. Thompson, The Royal Society of Chemistry, Analyst, 127, 1359-1364, (2002)
11   W. Horwitz and R. Albert, J. AOAC Int, 79.3, 589-621, (1996)
12   Bernard Rosner, Percentage Points for a Generalized ESD Many-Outlier Procedure, Technometrics, 25(2), 165-172, (1983)
13   iis memo 2202: reproducibility of AZO Dyes in Leather/Footwear andTextile in iis PTs