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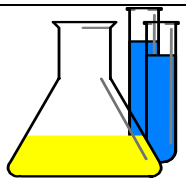

Validation of the Draft Community Reference Method for the determination of Solvent Yellow 124 in gas oil (Euromarker)

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Thomas Linsinger[1], Ger Koomen[2], Håkan Emteborg[1], Gert Roebben[1], Gerard Kramer[1], A. Lamberty[1]

1) DG-JRC, Institute for Reference Materials and Measurements, Retieseweg 111, 2440 Geel (BE)

2) Dutch Customs Laboratory, Kingsfordweg 1, 1043GN Amsterdam (NL)

1 EXECUTIVE SUMMARY

12 batches of samples containing various levels of solvent yellow 124 were prepared. The samples were sent to 26 different laboratories in order to validate the new Community Reference Method for the determination of Solvent Yellow 124 (Euromarker) in gas oil.

The interlaboratory trial was evaluated according to ISO 5725 and ISO 4259 for determination at 410 and 450 nm. After removal of outliers, repeatability and reproducibility standard deviations were calculated. At the level of 0.12 mg/L the repeatability standard deviation and the between laboratory standard deviation are of the same order. Reproducibility standard deviation at 0.12 mg/L is in the order predicted by the Horwitz equation (0.021 mg/L). The reproducibility standard deviation at 6 mg/L (batch 2) was with 0.23 mg/L better than the 0.4 mg/L that was thought to be achievable. Based on these standard deviations, limits for exceeding the specifications of 6 and 9 mg/L were calculated to be < 5.6 and > 9.5 mg/L respectively.

2 INTRODUCTION

2.1 Background

In its meeting of April 3 and 4 2003, the Excise Committee agreed to set-up an ad-hoc working group. In the mandate of the ad-hoc working group the following task was described:

The group's task is to look at ways of improving and if necessary harmonising the methods of analysis to be used for the detection of the Euromarker, and the determination of its marking levels with particular reference to:

a) *Explore whether method 455 MAD, Rev.1 (HPLC) for laboratory analysis provides a solid basis for developing a harmonised Community reference method of analysis for the Euromarker, Solvent Yellow 124 (SY 124), as requested by the Excise Committee;*

b) *Explore the conditions under which this method can also be used for identifying SY 124, if other national markers are used in parallel with the Euromarker;*

c) *Identify improvements to be made to this method of analysis, the procedure to follow and the time needed, in order to provide reliable and comparable data, which correspond to international standards, for the interpretation of the results;*

d) *Consider whether the precision performance of method 455 MAD, Rev.1 (HPLC) is in line with the principles of ISO 4259. In this context the reproducibility value to use in the reference method should be established at Community level based on the results of round robin tests. The experience gained during the Euromarker selection process within the JRC Ispra, or by Member States which have developed their national methods of analysis, should allow the Working Group Members to establish synergies and horizontal co-operation among themselves and to push this exercise through within reasonable time limits;*

e) *Explore whether it is really necessary to establish a mandatory operational mode or to merely establish a reference method that leaves Member States free to apply the latter in accordance with the principle of subsidiarity;*

f) *Consider the possibility to improve the comparability of the results of tests by determining reference materials, certified and provided by IRMM, and providing statistical data;*

g) *Explore whether method 455 MAD, Rev.1 causes problems in practice from environmental and health points of view, and provide solutions to be applied by all Member States.*

h) *Allow for efficient and reliable updates;*

i) *Establish all necessary conditions in carrying out the tasks agreed by the Excise Committee*

2.2 Method of analysis

The working group concluded in its first meeting at 22.10.2003 that improvements were needed for the method of analysis used by the Member States to detect the common fiscal marker solvent yellow 124 as provided for by Directive 95/60/EC of 27 November 1995 (OJ L291, of 06.12.1995,p. 46) and the Commission Decision 2001/574/EC of 13 July 2001 (OJ L203, of 28.07.2001,p. 28).

In the period from October 2003 to February 2004 all member states got the possibility to give their opinion about the optimal HPLC method. This resulted in the method that is used in this method evaluation (ANNEX D). The method is based on the method called 455MAD, Rev.1 (HPLC). The major provisions are:

- The calibration solutions are made in Xylene.
- A 3 point calibration curve is prescribed which is forced through zero and measured in duplicate.
- The retention time of SY124 has to be between 2 and 4 times the retention time of the void volume (t0) and the relative standard deviation of the retention time should be less then 2 %.
- The UV-detection is performed both on 410 nm and 450 nm.

The following targets were defined for the study:

- Repeatability standard deviations should be evaluated.
- Reproducibilities at concentrations of 0.12 and 6 mg/L should be not higher than 0.06 mg/L and 1.2 mg/L, respectively. These values correspond to reproducibility standard deviations of 0.021 mg/L and 0.42 mg/L respectively[1]. The value for the 0.12 mg/L level was chosen using the Horwitz equation, whereas for 6 mg/L a better reproducibility than predicted by Horwitz was deemed possible.
- If possible, no false positive results.

The method used by the participants is given in Annex D. Small changes (limit of detection, uncertainty) were made based on the evaluation of the validation study. The final method (ANNEX F) will be published in the "C Series" of the Official Journal of the European Union.

2.3 Matrix materials

To explore whether national dyes and markers possibly interfere with the SY124 determinations several national dyes and markers are added in known and realistic concentrations to the fuels under investigation. Also known concentrations of SY124 were added. In total 12 different compositions were defined and included in the round-robin.

2.4 Environmental aspects of the method.

The method investigated in this study can only be performed in a professional laboratory. Only the standard organic solvents toluene, xylene and ethyl acetate are used. These chemicals are less toxic than the gas oils that are analysed. All used solvents and analysed gas oils should be discarded as chemical waste.

[1] Reproducibility = $2 \cdot \sqrt{2} \cdot$ reproducibility standard deviation

3 ORGANISATION

The course of events is shown in Table 1.

Table 1: Timing of the study

Preparation of samples	March 2004
Dispatch of samples	last samples dispatched on 24.3.2004
Homogeneity study	12.5.2004
Sending of Draft report	18.5.2004
Evaluation meeting	4.6.2004

All laboratories received two samples of each batch, one from the first half of each batch, the other from the second half. Together with the samples, the laboratories received detailed instructions on the analyses to be performed and a reporting sheet. Duplicate analyses on each sample, i.e. four results per batch were to be performed. If possible, laboratories should report results at a determination wavelength of 410 and 450 nm.

The results were submitted to DCL, which forwarded them to IRMM for evaluation.

4 SAMPLES

4.1 Preparation

The preparation of the samples is described in more detail elsewhere [1]. 9 different commercial gas oils were delivered to IRMM by DCL. Characteristics of the materials are shown in Table 2.

Table 2: Characteristics of the fuels used. Methods: density: ASTM D4052; distillation: ASTM D86; SY124: reference method as described in Annex D; sulfur content: ISO/DIS 20486

batch	Density [kg/m^3]	Percentage distilling at 250°C	Percentage distilling at 350°C	SY124 [mg/L]	Sulfur content [mg/kg]	Colour
1,2,3	829.4	33	> 85	0.00	45.0	colourless
4	833.8	40	>85	0.00	45.6	colourless
5	831.8	42	>85	0.00	32.6	colourless
6	831.7	36	>85	0.00	45.6	colourless
7	832.7	38	>85	0.00	40.9	colourless
8,9	847.4	30	89	0	1900	colourless
10	831.2	38	>85	0.00	38.2	colourless
11	797	>65	>85	0.00	115	colourless
12	847.7	41	>85	>5	1967	red

Pure SY124 was obtained from John Hogg Technical Solutions Ltd, Manchester (UK). Purity was checked by DCL using HPLC and DSC and was found to be above 99 %. Dyeguard red 161 (purity 50 %), Dyeguard Red C (purity 60 %), Dyeguard Blue 79R (purity 48 %), Solvent Red 24 (purity > 90 %), Quinizarin (purity 100 %), Dyeguard green DL (33) (purity 74 %) and Coumarine (purity 100 %) were obtained from John Hogg technical solutions.

It should be noted that the SY124 used for the preparation of the samples was from a different supplier than the one sent to the participants for calibration. The latter had a purity of 92 % and was obtained from BASF. The results from the participants were corrected for the purity of this calibrant.

Stock solutions of the individual dyes in gas oil with concentrations ranging from 100 to 700 mg/kg were prepared. Pure gas oil was subsequently spiked with these stock solutions. The amount of gas oil used was not determined volumetrically but by weighing, as this gives a smaller uncertainty. The spiked gas oils were stirred with PTFE paddles before they were transferred into an automatic

ampouling machine. 2-mLsamples were filed into 3-mL ampoules, flushed with an Ar/He mixture and sealed. Tightness of the sealing was confirmed with a He-leak detector.

As the samples have been prepared by weighing, very accurate concentrations can be calculated. Uncertainties of these concentrations were estimated taking into account uncertainties from the preparation of the stock solutions and from the weighing of the gas oil. The composition of the samples, the concentration of SY124 and the uncertainties associated with these concentrations are shown in Table 3.

Table 3: Gravimetric addition of dyes to the various samples. U is the expanded uncertainty (k=2) for a 95 % confidence limit

Material	Gas oil	Other dye added		SY124 added	
		Substance	Conc. [mg/L]	Conc. [mg/L]	U [mg/L]
Batch 1	M-1919			0	-
Batch 2	M-1919			6.01	0.01
Batch 3	M-1919			0.12	0.00
Batch 4	M-2618	Solvent Red C	0.223	0.279	0.001
Batch 5	M-2619	Solvent Red C	4.78	5.96	0.02
Batch 6	M-2620	Dyeguard Blue 79R	4.98	8.97	0.03
Batch 7	M-2621	Solvent Red 24 Quinizarin	10.09 1.97	4.77	0.02
Batch 8	I-2882	Solvent Red C	0.199	0	-
Batch 9	I-2882	Solvent Red 161	15.0	6.02	0.03
Batch 10	M-2622	Dyeguard green DL(33)	4.91	7.19	0.03
Batch 11	I-2881	Coumarine	2.04	5.95	0.10
Batch 12	M2185			unknown	-

The uncertainties in Table 3 confirm the expected low uncertainty of the material property values.

4.2 Homogeneity

Ensuring the homogeneity of the samples is crucial for the suitability of materials for an interlaboratory comparison.

10 units of each batch were selected randomly stratified over the whole batch and analysed in duplicate. The results were plotted against the filling sequence to check for any significant trends. Standard deviations within units and between units were calculated using ANOVA. Furthermore, u^*_{bb}, the maximum heterogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [2]. The results of these evaluations are shown in Table 4.

Table 4: Results of the homogeneity test. n.c.= cannot be calculated as MSbetween < MSwithin

	s_{within}	$s_{between}$	u^*_{bb}	Slope significant (p= 95 %)
Batch 1	not applicable. No peaks were found.			
Batch 2	0.14%	0.10%	0.07%	no
Batch 3	2.20%	1.22%	1.04%	no
Batch 4	0.96%	n.c.	0.47%	no
Batch 5	0.20%	0.09%	0.10%	yes
Batch 6	0.24%	n.c.	0.11%	no
Batch 7	0.31%	0.64%	0.15%	yes
Batch 8	0.63%	0.43%	0.30%	no
Batch 9	1.47%	1.02%	0.70%	no
Batch 10	0.14%	0.61%	0.34%	yes
Batch 11	0.22%	0.13%	0.10%	no
Batch 12	0.81%	0.71%	0.38%	no

A significant trend was visible for the results of batch 5, 7 and 10. For batch 5 and 7, this trend only depends on the first data-point and is therefore likely to be due to an analytical artefact. For batch 10, the results ranged from 6.76 to 6.93, thus clearly indicating a trend in the filling sequence. However, this trend is small enough (maximum deviation < 1.2 %) to be negligible compared to the between laboratory standard deviation.

The trend was not significant for all other batches. Standard deviations between units range from 0.10 % to 1.2 %, even for batch 8, where a small positive result was found, although no SY124 was added. It can therefore be concluded that the materials are sufficiently homogeneous to be used for the method validation.

4.3 Stability

Stability of the SY124 in solution was assessed from samples stored at room temperature in the dark at the Dutch Customs Laboratory. Gas oil at a concentration of 6 and 0.12 mg/L was tested at 15 and 20 occasions, respectively over a period of 8 months without any indication of a significant degradation (confidence level 95 %). It was therefore concluded that the risk of degradation during the period of the study was negligible.

Additional confirmation of the stability of the sample can be derived from data of the homogeneity study, because these samples were analysed after the closing date for the interlaboratory study. The results obtained confirm the stability of the material over the time of the study.

4.4 Conclusion

Samples were prepared with a precisely known addition of SY124 and other dyes. Homogeneity was tested and the uncertainty of heterogeneity was found negligible compared to the between laboratory standard deviation. Stability was tested on similar materials and the results show that the danger of degradation during the time of the interlaboratory comparison is negligible. The materials are therefore well suited for an evaluation of the method.

5 RESULTS AND EVALUATION

All evaluations were done for results obtained by HPLC separation and UV detection at 410 nm and 450 nm using the draft reference method as described in Annex D and based on 455MAD, Rev.1 (HPLC).

The evaluation was done according to ISO 5725 "Accuracy (trueness and precision) of measurement method and results" [3] and ISO 4259 "Petroleum products - Determination and application of precision data in relation to methods of test" in its new draft version [4].

25 of the 26 laboratories submitted results in time. One of these 25 laboratories (Laboratory 3) used a different method than the common reference method (described in Annex D) and was therefore excluded from the evaluation. All laboratories were assigned anonymous codes to ensure confidentiality. All results are summarised for the individual batches in Annex A.

Results from batch 1 and 8 were excluded from several evaluations (outliers, bias, repeatability/reproducibility), because no SY124 was added. The target value was therefore zero, which makes evaluations impossible.

5.1 Outliers

The following tests were applied:

1. The data were first subjected to the Cochran test [3] at a 99 % confidence level to screen for outlying variances. Outlying variances were discarded and the test was repeated until no more outliers were found.
2. The Hawkins-test [4] at a 99 % confidence level was applied to the laboratory averages of the four results for each batch after the elimination of outliers of variance to check for outlying laboratory averages.

   Note: This test criterion tests the highest absolute deviation from the average of a particular sample. For the same relative precision, deviations will be higher at higher

concentrations than at lower concentrations. Results that were (visually) perfectly within the population at the high concentration level were marked as outliers, whereas clear outliers at the low concentration levels were not. Therefore, it was decided to restrict the test procedure to laboratory averages.

3. Laboratory averages were screened visually for outliers and deviations from a normal distribution using a normal probability plot [5].
4. The data sets after elimination of outliers were subjected to the graphical consistency technique (Mandel's h and k-plots [3]) to test for trends over all samples.

The total number of outliers under points 1-3 was usually restricted to three results, as both ISO 5725 and ISO 4259 suggest that not more than about 10 % of results should be discarded. Especially the Cochran test would have frequently resulted in higher numbers of outliers. The data were inspected visually in those cases and a decision on judgement was taken. The general tendency in this case was to keep datasets if there was no obvious difference to other datasets that would be kept.

The results that were eliminated because of the outlier tests are shown in Table 5. The reasons for elimination and the sequence of elimination are listed in Annex B together with the resulting normality plots and the plots from Mandel's h and k-statistics.

Looking at the results of Mandel's h-statistics (between laboratory consistency) and especially Mandel's k-statistics (within laboratory consistency) for results obtained at 410 nm, it is clear that Laboratory 24 delivered a vast fraction of results were h or k-values exceeded the warning or alarm level. This points at general problems for this type of measurement in this laboratory and its results were eliminated from the evaluation completely. For the determinations at 450 nm, no such clear trend was visible for any laboratory (Laboratory 24 did not submit results for 450 nm), therefore no dataset from any laboratory was eliminated completely for determinations at 450 nm. It is also clearly visible that a number of laboratories have a tendency towards either low or high results, which increased the reproducibility standard deviation. Nevertheless, these results were kept.

Table 5: Number of discarded results for both determination wavelengths

	Determination at 410 nm	Determination at 450 nm
Batch 2	3 (Laboratories 21, 24, 25)	1 (Laboratory 25)
Batch 3	2 (Laboratories 21, 24)	3 (Laboratories 11, 17, 21)
Batch 4	1 (Laboratory 24)	3 (Laboratories 13, 15, 21)
Batch 5	3 (Laboratories 5, 18, 24)	2 (Laboratories 11, 13)
Batch 6	1 (Laboratory 24)	2 (Laboratories 17, 21)
Batch 7	3 (Laboratories 1, 17, 24)	1 (Laboratory 1)
Batch 9	4 (Laboratories 1, 9, 15, 24)	3 (Laboratories 1, 2, 15)
Batch 10	3 (Laboratories 1, 14, 24)	2 (Laboratories 1, 14)
Batch 11	3 (Laboratories 1, 6, 24)	0
Batch 12	3 (Laboratories 2, 12, 24)	1 (Laboratory 14)

As can be seen in Table 5, total numbers of outliers do not differ significantly between wavelengths. The numbers of outliers therefore give no indication whether one wavelength is more appropriate for determination than the other.

Data for batch 3 (lowest SY124 content) at 450 nm were not normally distributed even after the outlier elimination procedure.

5.2 Repeatability and reproducibility

Repeatability (within-laboratory variance, s_r), between-laboratory variance (s_L) and reproducibility (s_R) were estimated using one-way analysis of variance as described in ISO 5725.

The data for repeatability and reproducibility at both determination wavelengths are shown in Table 6 and Table 7.

Table 6: Mean of means, repeatability standard deviation (s_r), between laboratory standard deviation (s_L) and reproducibility standard deviation (s_R) for the various batches for determination at 410 nm.

	Mean of means [mg/L]	s_r [mg/L] ([%])	s_L [mg/L] ([%])	s_R [mg/L] ([%])
Batch2	6.07	0.035 (0.58%)	0.214 (3.5 %)	0.217 (3.6 %)
Batch3	0.12	0.009 (7.00%)	0.011 (8.9 %)	0.014 (11.3 %)
Batch4	0.279	0.025 (8.82%)	0.023 (8.2 %)	0.034 (12.0 %)
Batch5	6.02	0.040 (0.67%)	0.187 (3.1 %)	0.191 (3.2 %)
Batch6	9.05	0.061 (0.68%)	0.246 (2.7 %)	0.254 (2.8 %)
Batch7	4.78	0.045 (0.93%)	0.138 (2.9%)	0.145 (3.0 %)
Batch9	6.12	0.051 (0.83%)	0.248 (4.1 %)	0.253 (4.1 %)
Batch10	7.16	0.070 (0.98%)	0.194 (2.7 %)	0.206 (2.9 %)
Batch11	5.87	0.044 (0.76%)	0.170 (2.9 %)	0.176 (3.0 %)
Batch12	6.10	0.092 (1.5 %)	0.361 (5.9 %)	0.373 (6.1 %)

Table 7: Mean of means, repeatability standard deviation (s_r), between laboratory standard deviation (s_L) and reproducibility standard deviation (s_R) for the various batches for determination at 450 nm.

	Mean of means [mg/L]	s_r [mg/L] ([%])	s_L [mg/L] ([%])	s_R [mg/L] ([%])
Batch2	6.04	0.041 (0.68 %)	0.228 (3.8 %)	0.231 (3.8 %)
Batch3	0.12	0.007 (5.44 %)	0.015 (12.4 %)	0.016 (13.5 %)
Batch4	0.27	0.014 (5.00 %)	0.014 (5.2 %)	0.02 (7.2 %)
Batch5	5.99	0.033 (0.55 %)	0.222 (3.7 %)	0.225 (3.7 %)
Batch6	9.05	0.064 (0.71 %)	0.271 (3.0 %)	0.279 (3.1 %)
Batch7	4.78	0.049 (1.03 %)	0.145 (3.0 %)	0.153 (3.2 %)
Batch9	6.10	0.079 (1.29 %)	0.267 (4.4 %)	0.278 (4.6 %)
Batch10	7.13	0.070 (0.98 %)	0.198 (2.8 %)	0.21 (2.9 %)
Batch11	5.87	0.061 (1.05 %)	0.178 (3.0 %)	0.189 (3.2 %)
Batch12	6.01	0.032 (0.54 %)	0.193 (3.2 %)	0.196 (3.3 %)

At the level of 6 mg/L repeatability standard deviation is significantly smaller (on average a factor 4) than the reproducibility standard deviation, which is not unusual for chemical analysis. Probably the difference between reproducibility and repeatability is mainly caused by the difficulty of precisely diluting the standards for the calibration curve.

At the level of 0.12 mg/L the repeatability standard deviation and the between laboratory standard deviation are of the same order. This means that at this level variation is mainly gouverned by intrinsic method variation and cannot be improved by further standardisation. Reproducibility standard deviation at 0.12 mg/L is of the order predicted by the Horwitz equation (0.021 mg/L). The reproducibility standard deviation at 6 mg/L (batch 2) was with 0.23 mg/L better than the 0.4 mg/L that was thought to be achievable.

Reproducibility standard deviations of batches containing more than 4 mg/L were found to vary randomly between 0.15 and 0.28 mg/L. An average reproducibility standard deviation was calculated using the relative s_R of batches 2, 5, 6, 7, 9, 10, 11 and 12 according to the equation

$$\bar{s}_R = \sqrt{\frac{\sum s_{R,i}^2}{8}} = 3.52\,\%$$

The average relative reproducibility standard deviation between 4.8 and 9 mg/L is 3.5 %.

The results for the reproducibility of the evaluated method make it possible to calculate the testing margins according to ISO 4259 for determination at 450 nm. Upper and lower limits of the specification were then calculated as

$limit = c \pm \frac{0.84 \cdot R}{\sqrt{2}}$ with $R = 2\sqrt{2} \cdot c \cdot \bar{s}_R$. The resulting limits were 9.5 and 5.6 mg/L, which means that

- a measured value higher then 9.5 mg/L means the concentration of SY124 in the gas oil is significantly higher than 9 mg/L.
- a measured value lower than 5.6 mg/L means the concentration of SY124 in the gas oil is significantly lower than 6 mg/L.

ISO 5725 suggests establishing a formal relationship between concentration and variation. This formal relationship was established by making a regression of the repeatability and reproducibility standard deviations, respectively, against the mean of means. The regression lines were tested for significance using a t-test. The changes of repeatability and reproducibility standard deviations with concentration are shown in Figure 1.

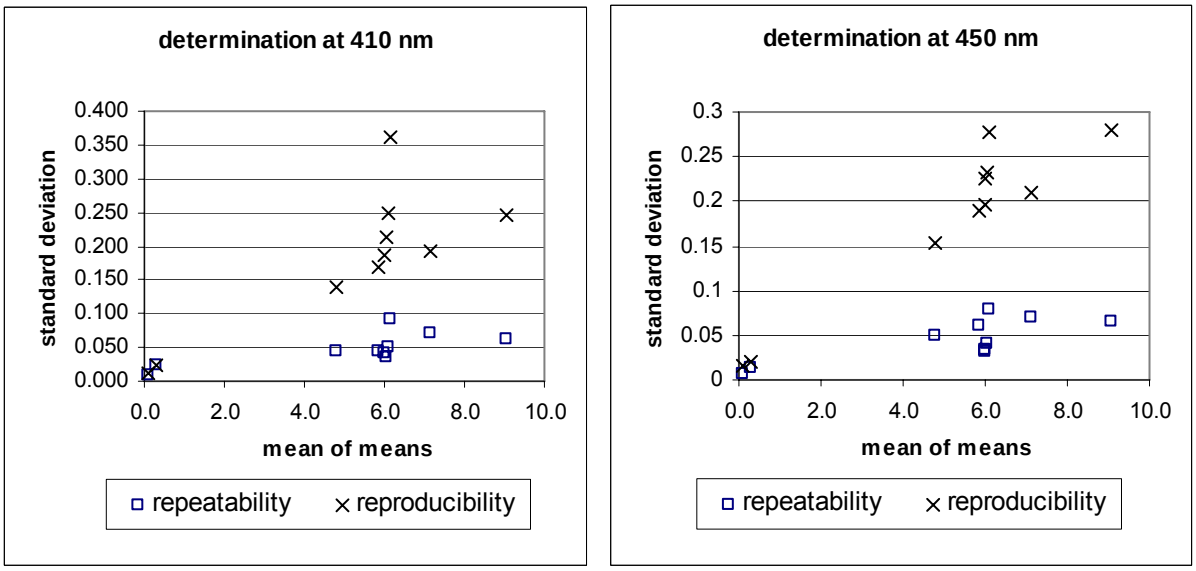

Figure 1: Concentration dependency of repeatability and reproducibility standard deviation

Repeatability and reproducibility standard deviations do not correlate with concentration in the range between 4.8 and 9 mg/L on a 95 % confidence level, as can be seen in Figure 1. Inclusion of the values of batches 3 and 4 makes the slopes of the regression lines significant. This significance, however, depends heavily on the data at the two lowest and the highest concentrations. With the current set of result, the correlation between the variations and the concentrations is therefore not reliable. Instead a constant relative reproducibility standard deviation of 3.5 % for detection at 450 nm was calculated for the relatively narrow concentration range of interest (5.6 - 9.5 mg/L)(see above).

Relative standard deviations are about constant down to 4 mg/L. As expected a much higher relative standard deviation is observed at the concentration level of 012 – 0,20 mg/L near the limit of quantification.

5.3 Bias

Method bias could be evaluated very accurately, as the samples were prepared by weighing which resulted in target values of an uncertainty that was negligible compared to the variation between

laboratories. The means of laboratory means were tested against this theoretical target value using a t-test. The results for both wavelengths are shown in Table 8.

Table 8: Comparison target value/mean of means for all batches and both determination wavelengths.

	Determination at 410 nm			Determination at 450 nm		
	Accepted sets of results	Target value [mg/L]	Mean of means [mg/L]	Accepted sets of results	Target value [mg/L]	Mean of means [mg/L]
Batch1	20	0.00	0.02	20	0.00	0.00
Batch2	21	6.01	6.07	19	6.01	6.04
Batch3	22	0.12	0.12	17	0.12	0.12
Batch4	23	0.279	0.279	17	0.279	0.272
Batch5	21	5.96	6.02	18	5.96	5.99
Batch6	23	8.97	9.05	18	8.97	9.05
Batch7	21	4.77	4.79	19	4.77	4.78
Batch8	20	0.00	0.131	20	0.00	0.00
Batch9	18	6.02	6.12	15	6.02	6.10
Batch10	21	7.19	7.16	18	7.19	7.13
Batch11	21	5.95	5.87	20	5.95	5.87
Batch12	21	not applicable	6.13	19	not applicable	6.01

For Batch 12, no target concentration can be given as it is a commercially available gas oil with an unknown SY124 content, contrary to the other batches where exactly known amounts of SY124 were added. None of the mean of means differs significantly from the target value (t-test; 95 % confidence level). For most batches, the means of laboratory means are above the target values. However, the deviation is small enough to contribute negligibly to measurement uncertainty. It therefore can be concluded that there is no significant method bias at both wavelengths used for determination.

5.4 Limit of detection and quantification

The limits of detection and quantification were determined via the standard deviation of the measurements under repeatability conditions of a sample with a concentration of SY124 near the limit of quantification. A small measurable analyte content is needed to be able to quantify a standard deviation. The standard deviation of batch 3 fulfils this criterion. As limit of detection and quantification are relevant to the individual laboratories rather than to a pooled group of laboratories, the repeatability standard deviations were used for the estimation of limit of detection and quantification.

The LOD was defined as three times the repeatability standard deviation of batch 3 following [6]. LOQ was arbitrarily defined as 10 times the repeatability standard deviation(see [6]). LODs and LOQs derived are shown in Table 9.

Table 9: LOD and LOQ for determination at 410 and 450 nm

	determination at 410 nm	determination at 450 nm
Limit of detection [mg/L]	0.026	0.020
Limit of quantification [mg/L]	0.085	0.065

False positive results

A target for the method is to make it possible to penalise drivers using fuel with a concentration of SY124 above 0.12 mg/L. It is therefore important that the method does not produce false positive results above 0.12 mg/L.

The results for batches 1 and 8 (no SY124 added) when measured at 410 nm are shown in Figure 2.

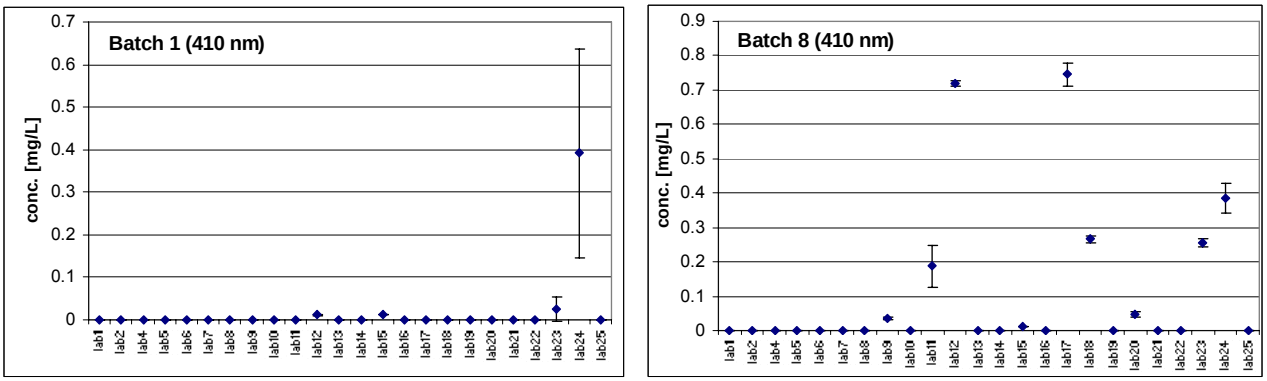

Figure 2: Results for the two blank materials when measured at 410 nm

As can be seen in Figure 2, for batch 1 only laboratory 24 delivered results above the LOD stated in Table 9. The situation was different for batch 8, where 8 laboratories delivered results above LOD (0.027 mg/L). Particularly worrying are results that are far above the minimally required LOQ of 0.12 mg/L. This indicates that the determination of extremely low levels of SY124 can be hampered by higher concentrations of other dyes. The laboratories were contacted to confirm the results. Laboratory 12 reported that the retention time of the peak evaluated was 2 % longer than in other chromatograms and therefore changed its results to "not detected". Laboratory 18 also reported distortion peaks and could therefore not confirm its positive results. Laboratory 20 reported a peak, but the UV spectrum was significantly different from SY124 and therefore stated that SY124 was not detected. Laboratory 24 reported that there was not enough sample to investigate the cause further. This discussion shows clearly that in commercial gas oils peaks may be present distorting determination at 410 nm. Careful investigation is required before a result is stated as exceeding the legal threshold.

The results for batches 1 and 8 when measured at 450 nm are shown in Figure 3.

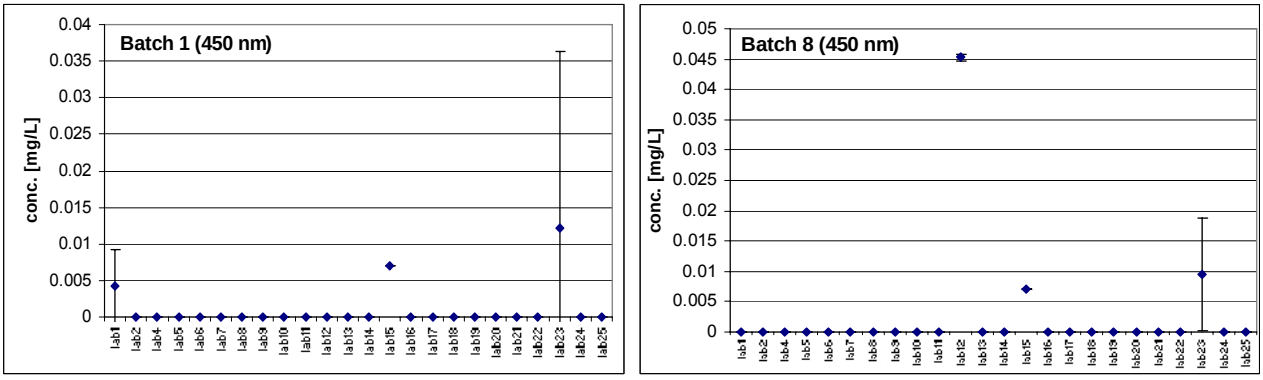

Figure 3: Results for the two blank materials when measured at 450 nm

Less false positive results for batch 8 were reported if the measurement is performed at 450 nm. As can be seen in Figure 3, only 3 laboratories reported false positives for batch 1 and batch 8. This indicates that determination at 450 nm is more robust against false positive results. Laboratory 1 stated that the positive result was a result of integrating noise, whereas also here the

retention time of the peak of laboratory 12 was 2 % longer than in the other chromatograms. Both positive results were therefore refuted. No information was obtained from laboratory 23.

No correlation of false positive results with the type of column used is visible. Columns that produced positive results in one laboratory did not do that in other laboratories (a complete list of the columns the participants used is given in Annex E)

Investigation of false positive results indicates that determination at 450 nm is less prone to delivering false positive results and should therefore be preferred if the gas oil contains solvent red C. No false positive results above 0.12 mg/L are reported at this detection wavelength.

Conclusion

The limits of detection and quantification are sufficiently low to allow quantification at a concentration level of 0.12 mg/L. Determination at 450 nm is less likely to deliver false positive results.

5.5 Ruggedness

Ruggedness of the method can be assessed using three sources:

- The reproducibility standard deviation gives a direct measure of the effects of slight laboratory-to-laboratory variations.
- Comparison of the repeatability and reproducibility standard deviations of the various batches. All batches were prepared with different concentrations of potentially influencing dyes and with different gas oils.
- Laboratory 3 used a method that deviated from the draft reference method. It applied internal standardisation with Pigment Yellow 3 and a gradient elution rather than isocratic conditions. The results were not included in the evaluation of the method but form a manner to check robustness of the method.

From the variation parameters (Table 6 and Table 7), it is obvious that different levels of potentially interfering substances do not influence repeatability and reproducibility. At a determination wavelength of 410 nm, 53 % of the variation in repeatability standard deviation and even 63 % of the differences in the reproducibility standard deviation can be explained by the differences in the SY124 concentration alone (values for 450 nm: 63 and 89 %), leaving very little room for influences of the presence and concentration of other dyes. It can therefore be concluded that the method is specific enough for SY124 without interference form other dyes present. Interfering peaks can pose problems especially at 410 nm, but can be identified via their retention time or UV spectrum.

The results from Laboratory 3 are compared with the overall averages in Table 10 to illustrate the influence of deviations from the prescribed method.

Table 10: Comparison of mean values of Laboratory 3 with target and mean of means values at 410 nm.

	Result lab. 3 [mg/L]	Target value [mg/L]	Mean of means [mg/L]
Batch 2	6.47	6.01	6.07
Batch 3	0.081	0.12	0.12
Batch 4	0.266	0.279	0.279
Batch 5	6.385	5.96	6.02
Batch 6	9.70	8.97	9.05
Batch 7	5.05	4.77	4.79
Batch 8	0.272	0	0.131
Batch 9	6.64	6.02	6.12
Batch 10	7.49	7.19	7.16
Batch 11	6.06	5.95	5.87
Batch 12	5.80	not applicable	6.13

For most batches, Laboratory 3 would have been among the other results, although there seems a tendency towards higher values (batch 2, 5, 6, 9, 11). This could be a systematic, laboratory inherent problem, as also a false positive result was found for batch 8. Also laboratory 2 analysed in a second run all samples with its in-house method using heptane/ethyl acetate 80/20, flow 2 mL/min and gave comparable results. These results indicate that the method is robust against modifications.

5.6 Quality criteria

Participants were requested to report the correlation coefficients of their calibrations. These might in the future be used to set acceptance criteria for a specific method. The correlation coefficients stated for the calibrations are shown in Table 11.

Table 11: Correlation coefficients

	Determination at 410 nm		Determination at 450 nm	
	Calibration 1	Calibration 2	Calibration 1	Calibration 2
Lab 1	0.99982	0.99982	0.9997	0.9997
Lab 2	0.99999		0.99998	
Lab 4	0.9999	0.9999	0.9999	0,9999
Lab 5	0.999820	0.999820	0.999370	0,999370
Lab 6	0.9998	0.9998	0.9995	0,9995
Lab 7	1	1		
Lab 8	0.99997	0.99997	0.99996	0,99996
Lab 9	>0.99	> 0.99		
Lab 10	0.9999	0.9999	1	1
Lab 11	0.9998	0.9998	0.9995	0,9995
Lab 12	0.9999	0.9999	1.0000	1,0000
Lab 13	0.9999		0.9996	
Lab 14	0.999542		0.999433	
Lab 15	0.9999		0.9999	
Lab 16	0.99996		0.99992	
Lab 17	0.999976	0.99976	0.999286	0,999286
Lab 18	0.999997	0.999999		
Lab 19	0.999996	0.99999	0.999983	0,999976
Lab 20	1.00000	1.00000	1.00000	1.00000
Lab 21	0.9999	09999	0.9998	0.9998
Lab 22	999936	0.999932	0.999793	0.999716
Lab 23	0.999956		0.999974	
Lab 24	0.9977	0.9977		
Lab 25	0.99936	0.99936	0.99864	0,99864

As can be seen in Table 11, most laboratories achieved correlation coefficients of 0.9990 or better. The fact that laboratory 24 was finally excluded from all evaluations due to an excessive number of outlying variances might hint to a connection to the fact that the correlation coefficients were the lowest stated. However, the amount of data is certainly not enough to draw a meaningful conclusion.

What can be concluded from the comparison of correlation data is that calibration as such does not pose a serious problem.

6 EVALUATION MEETING

An evaluation meeting was held on June 4 2004 to which all customs laboratories were invited. The outcome of the meeting was as follows:

- It was agreed that 450 nm was the preferred determination wavelength. Laboratories with a multi-wavelength detection system are of course free to use determination at 410 nm in addition.
- Some minor changes in the report were suggested which are included in this report.
- Participants were asked whether they had corrected their results for the purity of the standard. Three had not. Their results were subsequently corrected and the evaluation was repeated.

The minutes of the meeting will be published on the internal WebPages of the Excise Committee (Circa).

7 SUMMARY AND CONCLUSION

- 12 batches of laboratory inter-comparison samples for the validation of the Draft Community Reference Method for the determination of SY124 in gas oil were gravimetrically prepared and their homogeneity was determined.
- In the concentration range from 4 to 9 mg/L repeatability standard deviation at 410 nm and 450 nm, respectively are 0.056 and 0.056 mg/L. In this range the relative reproducibility standard deviations at 410 nm and 450 nm are 3.7 and 3.5 %. These reproducibility standard deviations are better than predicted by the Horwitz equation.
- At a concentration level of 0.12 mg/L repeatability standard deviation at 410 nm and 450 nm, are 0.009 mg/L (7.4 %) and 0.007 mg/L (5.4 %), respectively. Reproducibility standard deviations for determination at 410 nm and 450 nm are 0.015 mg/L (11.9 %) and 0.018 mg/L (14.9 %). These reproducibility standard deviations are better than predicted by the Horwitz equation.
- The method does not have any detectable bias at any of the two determination wavelengths. Very low concentrations of SY124 could be easier determined at 450 nm, because of the lower number of interfering peaks in the gas oils tested. Therefore, determination at 450 nm is preferable.
- The developed reference method does not cause problems in practice from environmental and health point of view as long as it is performed in a professional laboratory.
- The method will be published in the Official Journal of the European Union.

8 PARTICIPANTS

Project Coordination
Dutch Customs Laboratory, Amsterdam (NL), Mr. Koomen

Purity, homogeneity and stability measurements.

Dutch Customs Laboratory, Amsterdam (NL), Mrs Hovener, Mr Polmans, Mrs Sarneel, Mrs Zwaagstra, Mr Bongaerts

Sample preparation, statistical evaluation

Institute for Reference Materials and Measurements, Geel (BE), Mr. Emteborg, Mr, Linsinger, Mr. Roebben, Mr. Kramer, Ms. Lamberty

Interlaboratory comparison

Agenzia delle Dogane, Roma (IT), Mr. Massimo
BASF Aktiengesellschaft, Ludwigshafen (DE), Mr. Vamvakaris
Belastingsdienst/Douane Laboratorium, Amsterdam (NL), Mr. de Groot
Customs laboratory of National Customs Board, Riga (LV), Ms. Berna
Customs Technical Laboratory, Praha (CZ), Mr. Havelec
Finnish Customs Laboratory, Espoo (FI), Mr. Aholainen
Force Technology, Brondby (DK), Mr. Bjarnov
General Chemical State Laboratory, Athens (GR), Mr. Nomikos
John Hogg Technical Solutions, Manchester (UK), Mr. McCallien
Laboratoire des Douanes de Paris, Paris (FR), Mr. Audeon
Laboratoire National de Santé, Luxembourg (LU), Mr. Flies
Laboratorio Central de Aduanas, Madrid (ES), Mr. Iglesias
Laboratório da Direcão-Geral das Alfândegas, Lisboa (PT), Ms. Costa
Laboratorio de la Aduana, Barcelona (ES), Mr. Fernández
Laboratorium van Douane en Accijnzen, Leuven (BE), Mr. Van Bossuijt
Laboratory of the Government Chemist, Teddington (UK), Mr. Rattanjit

Lithuanian Customs Laboratory, Vilnius (LT), Ms. Morkuniene
National Laboratory of Forensic Science, Linköping (SE), Ms. Nilsson
Orgachim, Oissel (FR), Ms. Tesson
State Laboratory, Dublin (IRL), Mr. Mc.Gowan
Technische Untersuchungsanstalt, Wien (AT), Mr. Matiasovits
Zolltechnische Prüfungs- und Lehranstalt Berlin, Berlin (DE), Mr. Dering
Zolltechnische Prüfungs- und Lehranstalt Frankfurt, Frankfurt (DE), Mr. Dossmann
Zolltechnische Prüfungs- und Lehranstalt Hamburg, Hamburg (DE), Mr. Soldat
Zolltechnische Prüfungs- und Lehranstalt Köln, Köln (DE), Mr. Matthes
Zolltechnische Prufungs- und Lehranstalt München, München (DE), Mr. Peltzer

9 GLOSSARY

ANOVA ..... Analysis of variance
ASTM ..... American Society for Testing and Materials
DCL ..... Dutch Customs Laboratory
DSC ..... differential scanning calorimetry
IRMM ..... Institute for Reference Materials and Measurements
HPLC ..... high performance liquid chromatography
ISO ..... International Standardisation Organisation
ISO DIS ..... ISO Draft International Standard
IUPAC ..... International Union of Pure and Applied Chemistry
JRC ..... Directorate General Joint Research Centre
LOD ..... limit of detection
LOQ ..... limit of quantification
PTFE ..... polytetrafluoroethylene
SY124 ..... Solvent Yellow 124
s_r ..... repeatability standard deviation
s_R ..... reproducibility standard deviation
s_L ..... between laboratory standard deviation

10 ACKNOWLEDGEMENTS

The authors wish to acknowledge DG TAXUD for its financial support and all participants for their co-operation in this validation study.

11 ANNEXES

Annex A: Individual results for each batch for 410 nm and 450 nm; Graphical depiction of all results and normal probability plot of accepted results

Annex B: Sequence of outlier elimination; Plots of Mandel's h and k statistics

Annex C: z-scores for all laboratories, batches and wavelengths. z-scores are based on means and standard deviation of laboratory means. No z-scores were calculated for batches 1 and 8, as the target concentration for them is zero.

Annex D: Draft reference method for the determination of SY124 as distributed to the participants.

Annex E: Chromatographic columns used by the participants

Annex F: Reference method for the determination of SY124 as to be published in the Official Journal of the European Union.

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5 D.M. Levine, D. Stephan, T.C. Krehbiel, M. L. Berenson, Statistics for Managers using Microsoft Excel, Prendice Hall, New Jersey, 2002 *p. 243-248*

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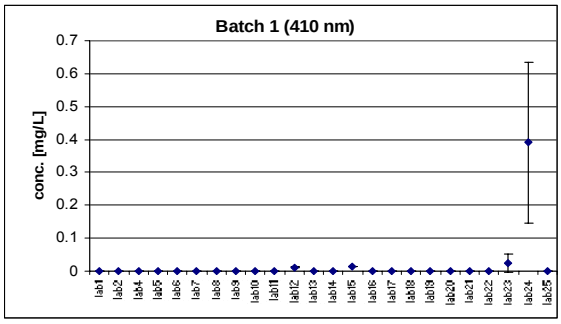
Batch 1, 410 nm

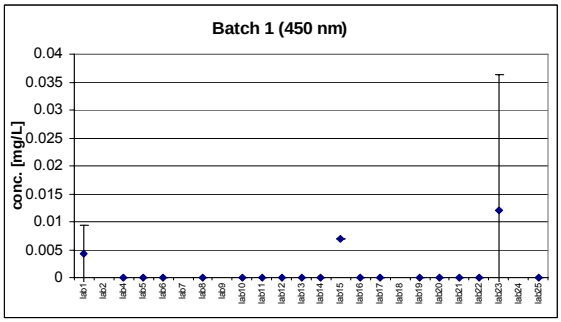
lab #	result 1	result 2	result 3	result 4
lab1	0.001	0	0	0
lab2	n.d.	n.d.	n.d.	n.d.
lab4	0	0	0	0
lab5	missing	missing	missing	missing
lab6	0	0	0	0
lab7	0	0	0	0
lab8	0	0	0	0
lab9	0	0	0	0
lab10	n.n.	n.n.	n.n.	n.n.
lab11	0	0	0	0
lab12	0.012	0.01	0.014	0.011
lab13	0	0	0	0
lab14	0	0	0	0
lab15	0.013	0.013	0.013	0.013
lab16	0		0	
lab17	0	0	0	0
lab18	0	0	0	0
lab19	0	0	0	0
lab20	0	0	0	0
lab21	0	0	0	0
lab22	0	0	0	0
lab23	0.00	0.00	0.055	0.0416
lab24	0.397	0.084	0.682	0.403
lab25	<0,05	<0,05	<0,05	<0,05



n.d.: not detectable

Batch 1, 450 nm

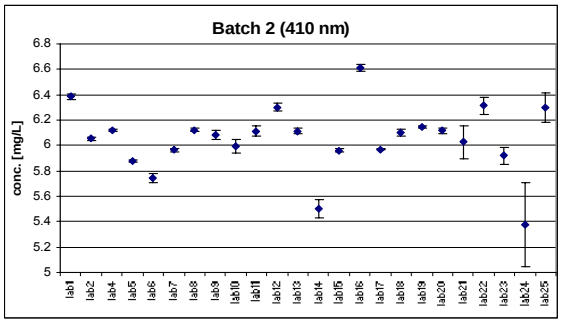
lab #	result 1	result 2	result 3	result 4
lab1	0	0	0.01	0.007
lab2	n.d.	n.d.	n.d.	n.d.
lab4	0	0	0	0
lab5	n.d.	n.d.	n.d.	n.d.
lab6	0	0	0	0
lab7	no results submitted			
lab8	0	0	0	0
lab9	no results submitted			
lab10	n.d.	n.d.	n.d.	n.d.
lab11	0	0	0	0
lab12	0	0	0	0
lab13	0	0	0	0
lab14	0	0	0	0
lab15	0.007	0.007	0.007	0.007
lab16	0		0	
lab17	0	0	0	0
lab18	no results submitted			
lab19	0	0	0	0
lab20	0	0	0	0
lab21	0	0	0	0
lab22	0	0	0	0
lab23	0	0	0.0483	0
lab24	no results submitted			
lab25	<0,05	<0,05	<0,05	<0,05

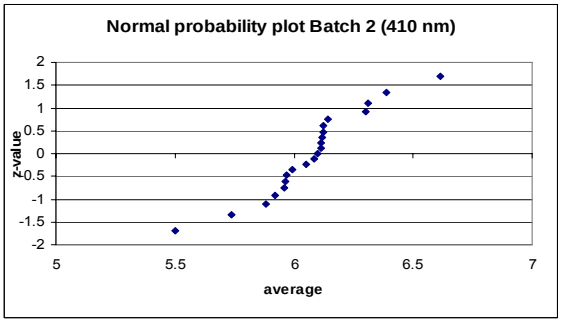


Shown are laboratory averages and standard deviations of all laboratories.

Batch 2, 410 nm (discarded results shaded)

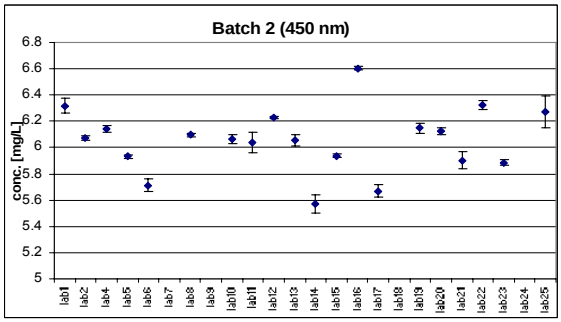
lab #	result 1	result 2	result 3	result 4
lab1	6.373	6.368	6.382	6.42
lab2	6.031	6.0546	6.067	6.058
lab4	6.113	6.127	6.128	6.115
lab5	5.878	5.895	5.878	5.869
lab6	5.715	5.788	5.709	5.7487
lab7	5.979	5.956	5.969	5.955
lab8	6.124	6.106	6.138	6.114
lab9	6.083	6.067	6.055	6.131
lab10	5.959	5.948	5.999	6.068
lab11	6.142	6.147	6.092	6.065
lab12	6.289	6.275	6.297	6.343
lab13	6.118	6.133	6.083	6.116
lab14	5.454	5.507	5.443	5.603
lab15	5.943	5.972	5.957	5.967
lab16	6.593		6.632	
lab17	5.968	5.973	5.979	5.964
lab18	6.000	6.000	6.000	6.000
lab19	6.145	6.144	6.133	6.156
lab20	6.114	6.144	6.091	6.123
lab21	6.089	5.848	6.027	6.148
lab22	6.3	6.331	6.389	6.23
lab23	5.9074	5.8829	6.0189	5.8732
lab24	5.618	5.621	4.916	5.345
lab25	6.182	6.416	6.378	6.209

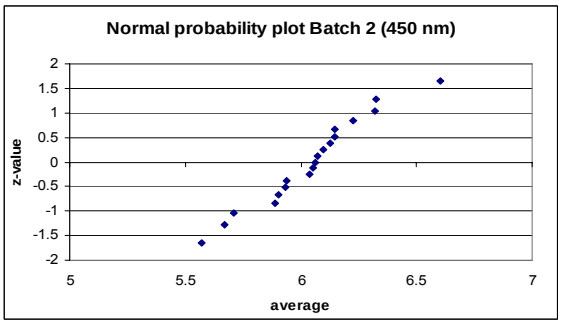




Batch 2, 450 nm (discarded results shaded)

lab #	result 1	result 2	result 3	result 4
lab1	6.269	6.314	6.292	6.401
lab2	6.062	6.055	6.086	6.082
lab4	6.136	6.183	6.129	6.129
lab5	5.926	5.954	5.929	5.923
lab6	5.686	5.771	5.668	5.724
lab7	no results submitted			
lab8	6.109	6.080	6.110	6.090
lab9	no results submitted			
lab10	6.074	6.085	6.075	6.010
lab11	6.119	6.030	5.930	6.063
lab12	6.228	6.238	6.223	6.223
lab13	6.040	6.115	6.011	6.049
lab14	5.506	5.622	5.523	5.638
lab15	5.922	5.949	5.936	5.946
lab16	6.597		6.613	
lab17	5.645	5.638	5.65	5.737
lab18	no results submitted			
lab19	6.091	6.159	6.174	6.164
lab20	6.119	6.165	6.105	6.119
lab21	5.900	5.846	5.873	5.996
lab22	6.306	6.335	6.366	6.291
lab23	5.907	5.873	5.899	5.867
lab24	no results submitted			
lab25	6.088	6.302	6.346	6.348



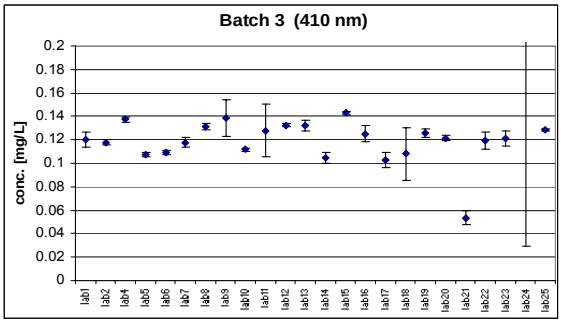


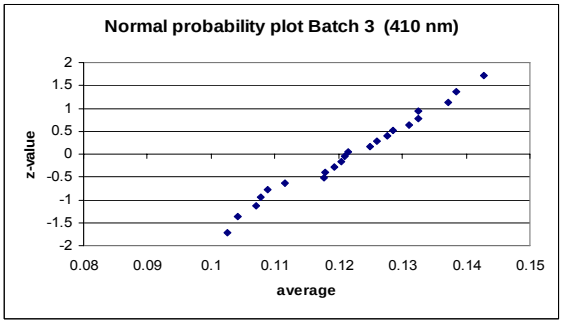
Shown are laboratory averages and standard deviations of all laboratories.

Normal probability plot: only accepted sets of results are shown

Batch 3, 410 nm (discarded results shaded)

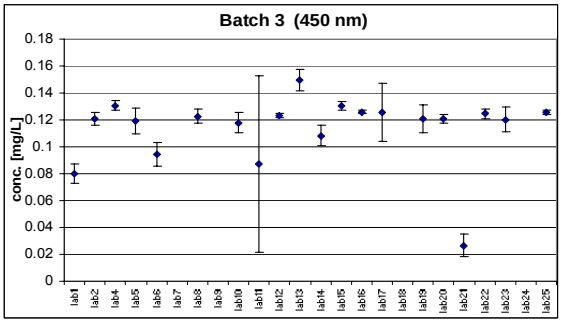
lab #	result 1	result 2	result 3	result 4
lab1	0.120	0.113	0.129	0.120
lab2	0.121	0.1166	0.1176	0.116
lab4	0.140	0.135	0.139	0.135
lab5	0.108	0.105	0.109	0.106
lab6	0.108	0.111	0.107	0.110
lab7	0.121	0.117	0.112	0.121
lab8	0.127	0.131	0.132	0.134
lab9	0.116	0.141	0.15	0.147
lab10	0.114	0.110	0.111	0.111
lab11	0.100	0.121	0.137	0.153
lab12	0.133	0.133	0.133	0.131
lab13	0.129	0.134	0.138	0.129
lab14	0.103	0.099	0.105	0.110
lab15	0.141	0.144	0.142	0.144
lab16	0.120		0.130	
lab17	0.107	0.107	0.093	0.103
lab18	0.100	0.130	0.080	0.121
lab19	0.128	0.123	0.130	0.123
lab20	0.120	0.124	0.119	0.123
lab21	0.054	0.06	0.055	0.045
lab22	0.125	0.123	0.109	0.120
lab23	0.120	0.129	0.122	0.113
lab24	0.837	0.300	0.167	0.127
lab25	0.127	0.130	0.128	0.129

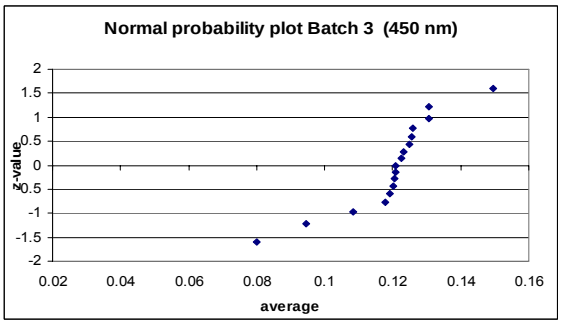




Batch 3, 450 nm (discarded results shaded)

lab #	result 1	result 2	result 3	result 4
lab1	0.089	0.076	0.082	0.073
lab2	0.127	0.117	0.117	0.122
lab4	0.134	0.13	0.133	0.126
lab5	0.13	0.125	0.113	0.109
lab6	0.098	0.099	0.081	0.099
lab7	no results submitted			
lab8	0.130	0.121	0.121	0.118
lab9	no results submitted			
lab10	0.127	0.112	0.121	0.112
lab11	0.115	0.155	0.080	0.00
lab12	0.124	0.124	0.121	0.124
lab13	0.161	0.144	0.147	0.145
lab14	0.119	0.107	0.101	0.106
lab15	0.134	0.126	0.132	0.130
lab16	0.125		0.127	
lab17	0.105	0.115	0.155	0.128
lab18	no results submitted			
lab19	0.129	0.110	0.131	0.114
lab20	0.118	0.120	0.119	0.125
lab21	0.031	0.014	0.031	0.030
lab22	0.123	0.13	0.122	0.124
lab23	0.108	0.127	0.120	0.126
lab24	no results submitted			
lab25	0.128	0.126	0.125	0.124



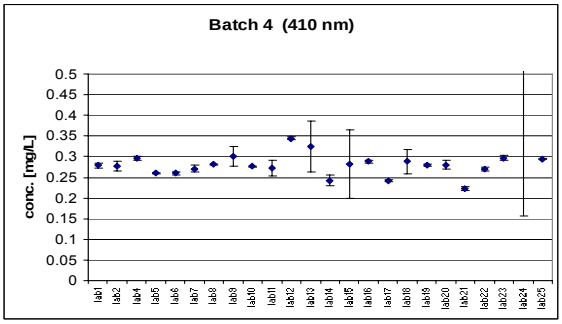


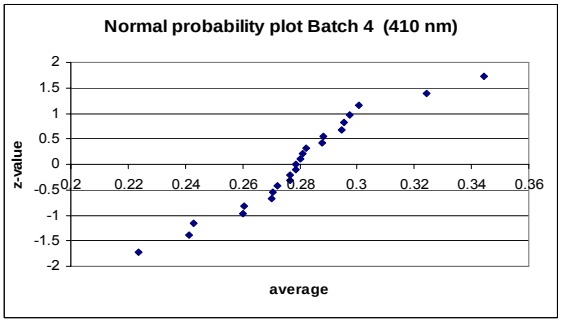
Shown are laboratory averages and standard deviations of all laboratories.

Normal probability plot: only accepted sets of results are shown

Batch 4, 410 nm (discarded values shaded)

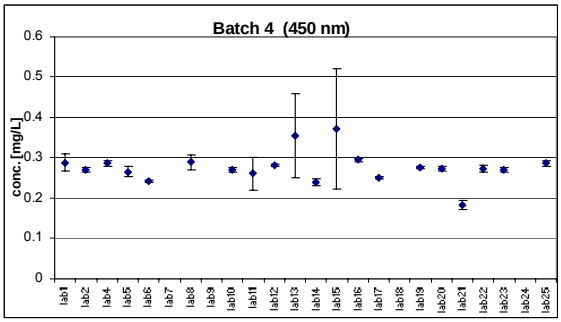
lab #	result 1	result 2	result 3	result 4
lab1	0.284	0.281	0.272	0.277
lab2	0.265	0.270	0.286	0.288
lab4	0.290	0.301	0.293	0.297
lab5	0.261	0.262	0.259	0.260
lab6	0.259	0.256	0.263	0.262
lab7	0.273	0.280	0.269	0.260
lab8	0.280	0.280	0.282	0.282
lab9	0.274	0.290	0.331	0.308
lab10	0.276	0.276	0.278	0.276
lab11	0.282	0.285	0.244	0.278
lab12	0.346	0.349	0.341	0.341
lab13	0.268	0.279	0.394	0.352
lab14	0.240	0.261	0.239	0.231
lab15	0.331	0.327	0.314	0.157
lab16	0.291		0.285	
lab17	0.239	0.243	0.243	0.241
lab18	0.251	0.296	0.283	0.322
lab19	0.281	0.28	0.276	0.278
lab20	0.268	0.285	0.292	0.275
lab21	0.219	0.23	0.224	0.222
lab22	0.27	0.269	0.278	0.264
lab23	0.3021	0.2899	0.299	0.298
lab24	1.006	0.496	0.26	0.265
lab25	0.293	0.296	0.295	0.295

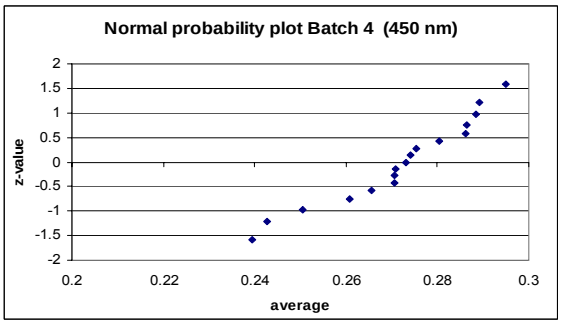




Batch 4, 450 nm (discarded values shaded)

lab #	result 1	result 2	result 3	result 4
lab1	0.304	0.309	0.271	0.27
lab2	0.268	0.278	0.268	0.268
lab4	0.278	0.285	0.287	0.295
lab5	0.281	0.251	0.263	0.267
lab6	0.240	0.244	0.240	0.247
lab7	no results submitted			
lab8	0.308	0.266	0.282	0.301
lab9	no results submitted			
lab10	0.274	0.274	0.264	0.271
lab11	0.238	0.312	0.272	0.220
lab12	0.281	0.284	0.28	0.277
lab13	0.272	0.258	0.441	0.451
lab14	0.236	0.230	0.240	0.252
lab15	0.298	0.297	0.595	0.298
lab16	0.298		0.292	
lab17	0.249	0.248	0.254	0.251
lab18	no results submitted			
lab19	0.275	0.273	0.279	0.275
lab20	0.266	0.276	0.281	0.269
lab21	0.173	0.173	0.198	0.189
lab22	0.280	0.278	0.261	0.277
lab23	0.272	0.269	0.263	0.2785
lab24	no results submitted			
lab25	0.28	0.28	0.294	0.292



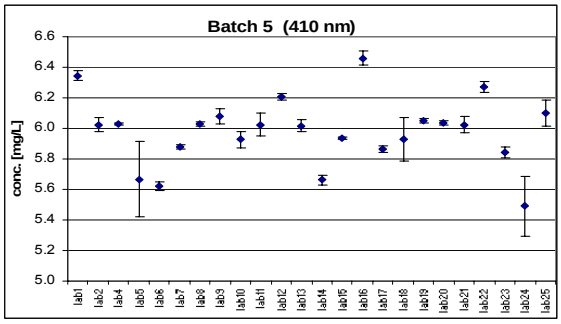


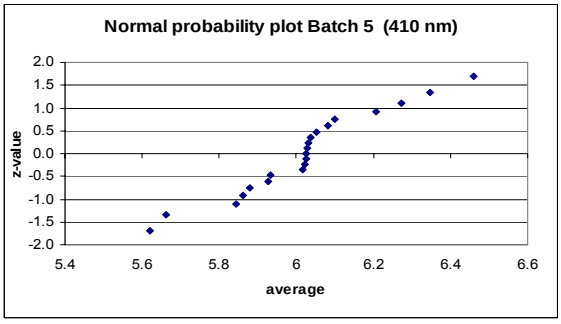
Shown are laboratory averages and standard deviations of all laboratories.

Normal probability plot: only accepted sets of results are shown

Batch 5, 410 nm (discarded results shaded)

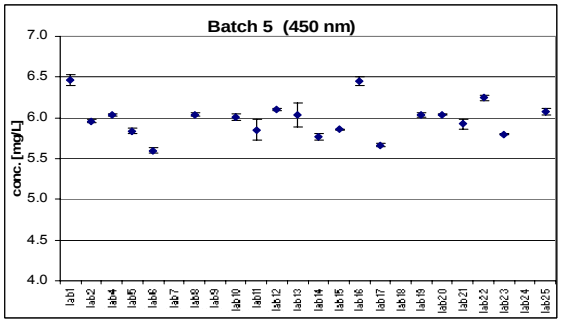
lab #	result 1	result 2	result 3	result 4
lab1	6.318	6.322	6.362	6.381
lab2	5.970	6.014	6.072	6.044
lab4	6.025	6.025	6.033	6.037
lab5	5.780	5.294	5.801	5.796
lab6	5.604	5.634	5.597	5.653
lab7	5.879	5.859	5.893	5.891
lab8	6.026	6.012	6.027	6.051
lab9	6.057	6.152	6.041	6.072
lab10	5.937	5.997	5.896	5.874
lab11	6.083	6.045	6.048	5.913
lab12	6.173	6.228	6.216	6.212
lab13	6.035	6.037	6.034	5.955
lab14	5.624	5.676	5.647	5.701
lab15	5.933	5.937	5.924	5.938
lab16	6.429		6.491	
lab17	5.889	5.837	5.859	5.865
lab18	6.050	5.745	6.033	5.879
lab19	6.053	6.067	6.032	6.059
lab20	6.038	6.030	6.025	6.051
lab21	6.001	6.038	5.966	6.092
lab22	6.251	6.242	6.315	6.279
lab23	5.898	5.828	5.826	5.826
lab24	5.576	5.699	5.237	5.451
lab25	5.976	6.133	6.113	6.179

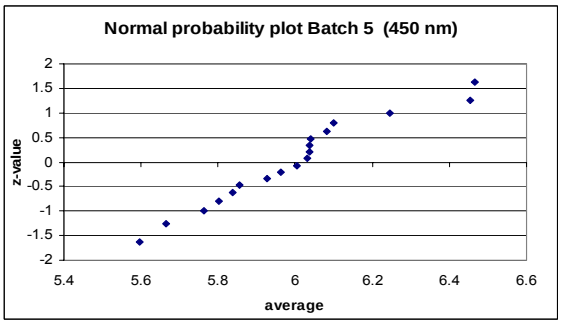




Batch 5, 450 nm (discarded results shaded)

lab #	result 1	result 2	result 3	result 4
lab1	6.42	6.452	6.561	6.434
lab2	5.954	5.942	5.976	5.974
lab4	6.036	6.025	6.047	6.036
lab5	5.788	5.843	5.868	5.852
lab6	5.582	5.606	5.562	5.634
lab7	no results submitted			
lab8	6.048	6.012	6.04	6.06
lab9	no results submitted			
lab10	6.059	5.976	6.013	5.968
lab11	5.861	5.732	6.026	5.793
lab12	6.088	6.092	6.099	6.117
lab13	6.160	6.134	5.986	5.842
lab14	5.710	5.779	5.766	5.804
lab15	5.864	5.848	5.865	5.85
lab16	6.418		6.490	
lab17	5.676	5.658	5.642	5.688
lab18	no results submitted			
lab19	6.048	6.058	6.002	6.016
lab20	6.034	6.033	6.033	6.047
lab21	5.869	5.993	5.884	5.962
lab22	6.229	6.218	6.285	6.247
lab23	5.804	5.807	5.805	5.788
lab24	no results submitted			
lab25	6.056	6.114	6.112	6.04



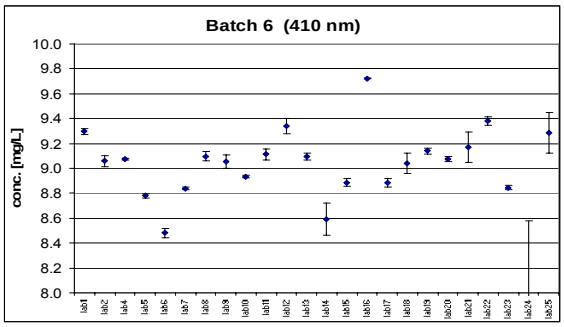


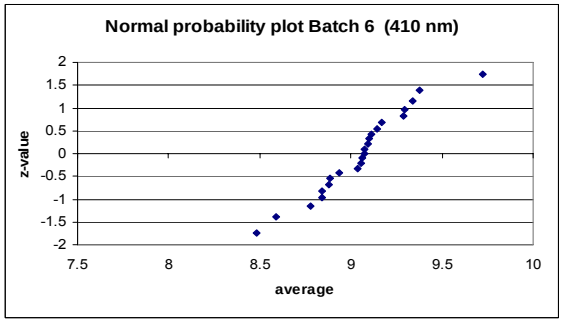
Shown are laboratory averages and standard deviations of all laboratories.

Normal probability plot: only accepted sets of results are shown

Batch 6, 410 nm (discarded results shaded)

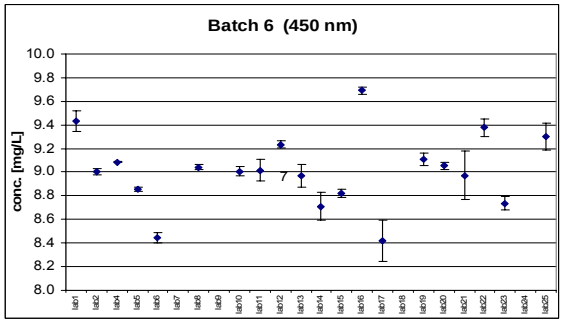
lab #	result 1	result 2	result 3	result 4
lab1	9.282	9.282	9.294	9.328
lab2	9.036	9.085	9.109	9.013
lab4	9.070	9.069	9.078	9.084
lab5	8.79	8.756	8.795	8.782
lab6	8.449	8.508	8.451	8.517
lab7	8.831	8.841	8.836	8.850
lab8	9.075	9.066	9.155	9.098
lab9	9.030	8.997	9.077	9.115
lab10	8.940	8.933	8.921	8.940
lab11	9.046	9.119	9.134	9.150
lab12	9.395	9.388	9.262	9.316
lab13	9.083	9.065	9.108	9.125
lab14	8.482	8.774	8.593	8.519
lab15	8.901	8.918	8.874	8.851
lab16	9.725		9.722	
lab17	8.839	8.889	8.921	8.875
lab18	9.125	8.987	9.091	8.954
lab19	9.107	9.150	9.147	9.164
lab20	9.103	9.061	9.059	9.074
lab21	9.130	9.287	9.012	9.240
lab22	9.387	9.424	9.347	9.359
lab23	8.821	8.861	8.851	8.842
lab24	8.402	8.557	7.261	7.632
lab25	9.327	9.396	9.048	9.372

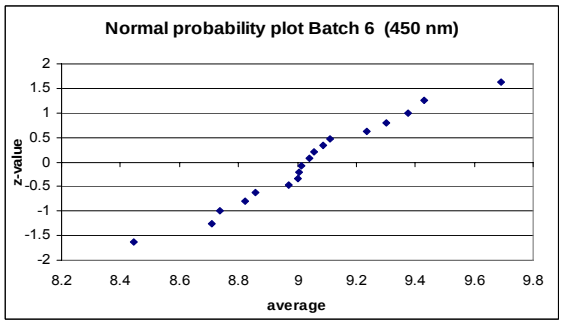




Batch 6, 450 nm (discarded results shaded)

lab #	result 1	result 2	result 3	result 4
lab1	9.360	9.361	9.536	9.463
lab2	8.975	8.993	9.0216	9.0247
lab4	9.092	9.083	9.085	9.086
lab5	8.879	8.842	8.858	8.853
lab6	8.399	8.480	8.411	8.484
lab7	no results submitted			
lab8	9.021	9.026	9.07	9.051
lab9	no results submitted			
lab10	9.002	8.951	9.039	9.037
lab11	8.884	9.024	9.068	9.086
lab12	9.266	9.253	9.218	9.198
lab13	9.024	8.879	9.072	8.901
lab14	8.597	8.871	8.715	8.664
lab15	8.857	8.793	8.837	8.797
lab16	9.713		9.670	
lab17	8.159	8.504	8.508	8.500
lab18	no results submitted			
lab19	9.093	9.048	9.148	9.16
lab20	9.061	9.075	9.011	9.072
lab21	8.892	9.187	8.727	9.083
lab22	9.382	9.472	9.285	9.365
lab23	8.708	8.692	8.723	8.821
lab24	no results submitted			
lab25	9.471	9.281	9.211	9.242



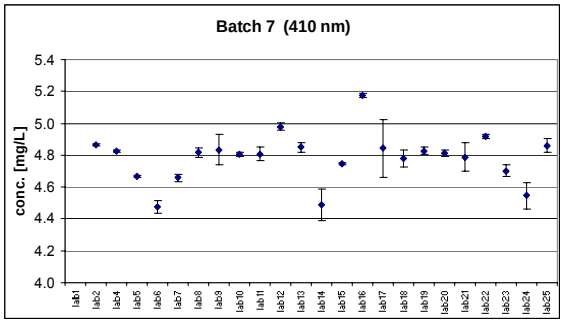


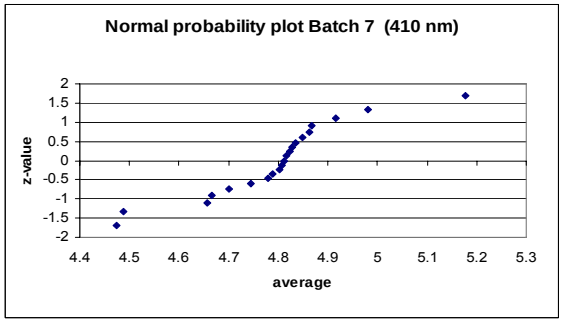
Shown are laboratory averages and standard deviations of all laboratories.

Normal probability plot: only accepted sets of results are shown

Batch 7, 410 nm (discarded results shaded)

lab #	result 1	result 2	result 3	result 4
lab1	3.227[1]	2.835[1]	2.924[1]	2.69[1]
lab2	4.860	4.863	4.868	4.877
lab4	4.831	4.822	4.82	4.824
lab5	4.665	4.656	4.671	4.676
lab6	4.421	4.464	4.497	4.513
lab7	4.649	4.632	4.672	4.676
lab8	4.796	4.789	4.845	4.837
lab9	4.819	4.714	4.868	4.939
lab10	4.785	4.811	4.813	4.805
lab11	4.790	4.772	4.804	4.866
lab12	4.980	4.953	5.008	4.979
lab13	4.849	4.833	4.826	4.890
lab14	4.568	4.386	4.580	4.417
lab15	4.740	4.741	4.744	4.757
lab16	5.167		5.187	
lab17	4.691	4.714	4.879	5.087
lab18	4.793	4.722	4.843	4.758
lab19	4.834	4.854	4.795	4.827
[1]**lab20**	4.782	4.811	4.828	4.825
lab21	4.861	4.756	4.861	4.675
lab22	4.914	4.936	4.910	4.903
lab23	4.668	4.671	4.727	4.739
lab24	4.665	4.522	4.527	4.466
lab25	4.840	4.863	4.823	4.921

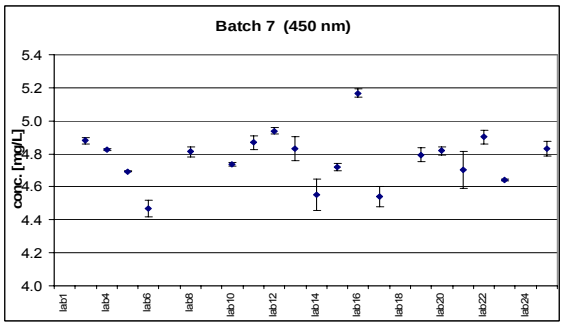


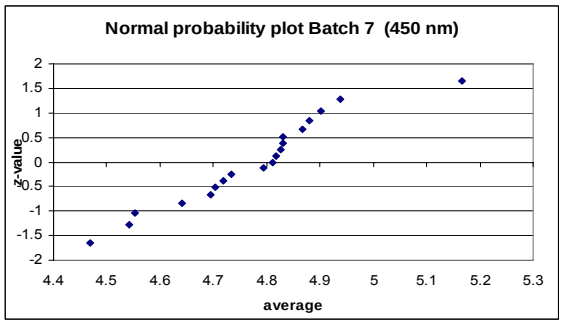


1) result in-house method: 4.528 mg/L

Batch 7, 450 nm (discarded results shaded)

lab #	result 1	result 2	result 3	result 4
lab1	3.312	2.831	2.981	2.604
lab2	4.876	4.852	4.889	4.901
lab4	4.832	4.821	4.83	4.825
lab5	4.692	4.696	4.693	4.696
lab6	4.398	4.498	4.477	4.506
lab7	no results submitted			
lab8	4.793	4.778	4.837	4.841
lab9	no results submitted			
lab10	4.727	4.738	4.748	4.723
lab11	4.890	4.877	4.806	4.898
lab12	4.930	4.917	4.953	4.957
lab13	4.800	4.937	4.817	4.767
lab14	4.632	4.458	4.636	4.482
lab15	4.693	4.738	4.708	4.739
lab16	5.184		5.150	
lab17	4.529	4.497	4.509	4.634
lab18	no results submitted			
lab19	4.800	4.759	4.770	4.850
lab20	4.783	4.823	4.837	4.830
lab21	4.751	4.709	4.806	4.548
lab22	4.841	4.937	4.925	4.903
lab23	4.6401	4.6318	4.651	4.643
lab24	no results submitted			
lab25	4.814	4.886	4.848	4.777



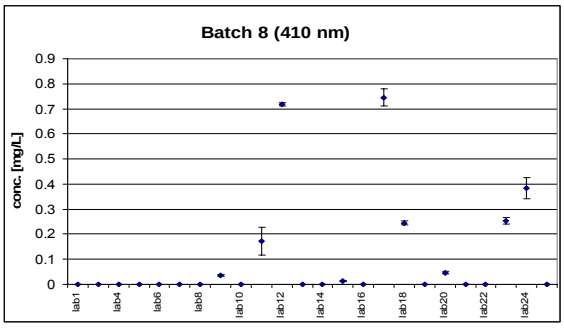


Shown are laboratory averages and standard deviations of all laboratories.

Normal probability plot: only accepted sets of results are shown

Batch 8, 410 nm

lab #	result 1	result 2	result 3	result 4
lab1	0	0.005	0.010	0
lab2	n.d.	n.d.	n.d.	n.d.
lab4	0	0	0	0
lab5	n.d.	n.d.	n.d.	n.d.
lab6	0[2]	0[2]	0[2]	0[2]
lab7	0	0	0	0
lab8	0	0	0	0
lab9	0.037	0.036	0.038	0.029
lab10	n.d.	n.d.	n.d.	n.d.
lab11	0.144	0.25	0.228	0.127
lab12	0.729	0.717	0.714	0.714
lab13	0	0	0	0
lab14	0	0	0	0
lab15	0.013	0.013	0.013	0.013
lab16	0	""	0	""
lab17	0.714	0.718	0.764	0.784
lab18	0.262	0.276	0.256	0.270
lab19	0	0	0	0
lab20	0.050[1]	0.0540[1]	0.040[1]	0.0440[1]
lab21	0	0	0	0
lab22	0	0	0	0
lab23	0.272	0.2497	0.255	0.242
lab24	0.43	0.374	0.403	0.331
lab25	<0,05	<0,05	<0,05	<0,05



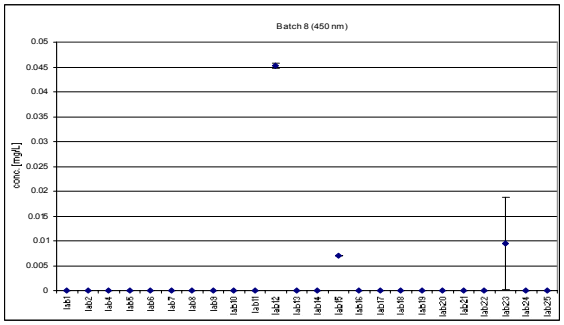
1) UV spectrum does not match the one of Solvent Yellow therefore should be "not detected"

2) The samples contain many other components that seem to stay very long in the system (the last elutiong about 45 min. after injection).

n.d.: not detectable

Batch 8, 450 nm

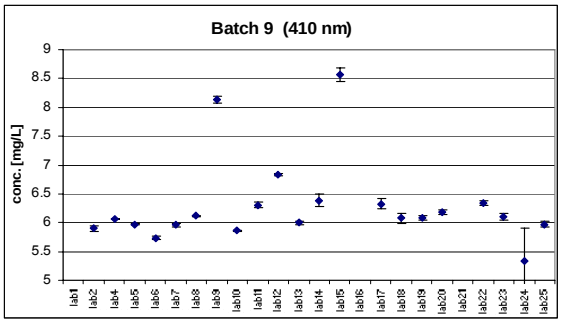
lab #	result 1	result 2	result 3	result 4
lab1	0	0	0	0.009
lab2	n.d.	n.d.	n.d.	n.d.
lab4	0	0	0	0
lab5	n.d.	n.d.	n.d.	n.d.
lab6	0	0	0	0
lab7	no results submitted			
lab8	0	0	0	0
lab9	no results submitted			
lab10	n.d.	n.d.	n.d.	n.d.
lab11	0	0	0	0
lab12	0.045	0.045	0.046	0.045
lab13	0	0	0	0
lab14	0	0	0	0
lab15	0.007	0.007	0.007	0.007
lab16	0		0	
lab17	n.d.	n.d.	n.d.	n.d.
lab18	no results submitted			
lab19	0	0	0	0
lab20	0	0	0	0
lab21	0	0	0	0
lab22	0	0	0	0
lab23	0.022	0.009	0	0.008
lab24	no results submitted			
lab25	<0,05	<0,05	<0,05	<0,05

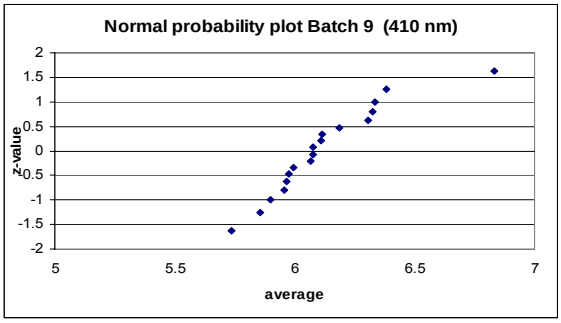


Shown are laboratory averages and standard deviations of all laboratories.

Batch 9, 410 nm (discarded results shaded)

lab #	result 1	result 2	result 3	result 4
lab1	2.158[4]	2.067[4]	2.455[4]	2.594[4]
lab2	5.818	5.907	5.934	5.924
lab4	6.068	6.066	6.062	6.066
lab5	5.954	5.968	5.993	5.982
lab6	5.779	5.705	5.715	5.748
lab7	5.932	5.924	5.981	5.995
lab8	6.106	6.123	6.119	6.114
lab9	8.210	8.159	8.128	8.068
lab10	5.843	5.867	5.874	5.85
lab11	6.358	6.335	6.259	6.281
lab12	6.832	6.848	6.827	6.816
lab13	5.979	6.030	5.995	5.985
lab14	6.471	6.236	6.454	6.361
lab15	8.405	8.607	8.672	8.568
lab16	9.390[3]		9.420[3]	
lab17	6.257	6.256	6.336	6.450
lab18	6.146	5.990	6.152	6.020
lab19	6.091	6.062	6.125	6.037
lab20	6.215[1]	6.213[1]	6.168[1]	6.144[1]
lab21	non reliable[2]			
lab22	6.336	6.278	6.376	6.349
lab23	6.148	6.058	6.060	6.167
lab24	5.121	6.189	5.027	5.017
lab25	5.959	6.042	5.930	5.941





1) Changed eluent concentration to 1 % ethyl acetate, which resulted in complete separation of a coeluting peak

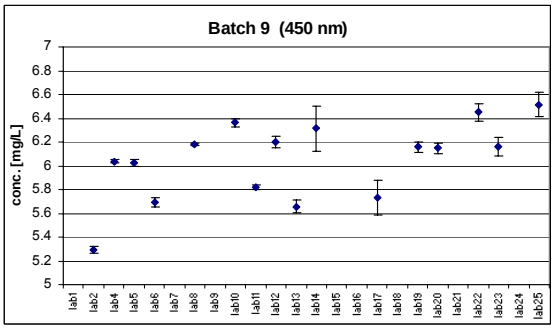
2) mixture of co-eluting markers

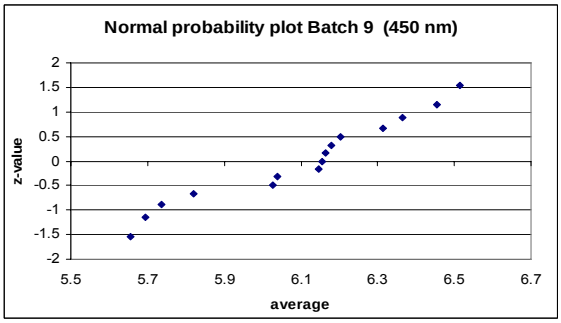
3) coelution with other compound,

4) result in-house method: 5.800 mg/L

Batch 9, 450 nm (discarded results shaded)

lab #	result 1	result 2	result 3	result 4
lab1	2.196	2.094	2.491	2.559
lab2	5.261	5.271	5.322	5.318
lab4	6.044	6.024	6.035	6.050
lab5	6.012	6.061	6.041	5.990
lab6	5.729	5.724	5.660	5.666
lab7	no results submitted			
lab8	6.173	6.191	6.18	6.170
lab9	no results submitted			
lab10	6.395	6.388	6.357	6.319
lab11	5.798	5.844	5.829	5.812
lab12	6.223	6.263	6.165	6.163
lab13	5.640	5.657	5.600	5.729
lab14	6.256	6.065	6.478	6.452
lab15	21.156	21.137	21.107	21.194
lab16	9.39[3]		9.42[3]	
lab17	5.673	5.612	5.712	5.945
lab18	no results submitted			
lab19	6.170	6.173	6.193	6.090
lab20	6.179	6.182	6.139	6.089
lab21	non reliable[2]			
lab22	6.426	6.544	6.471	6.372
lab23	6.238	6.178	6.1835	6.053
lab24	no results submitted			
lab25	6.375	6.578	6.510	6.600



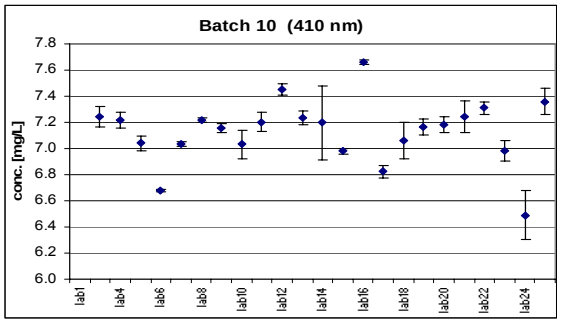


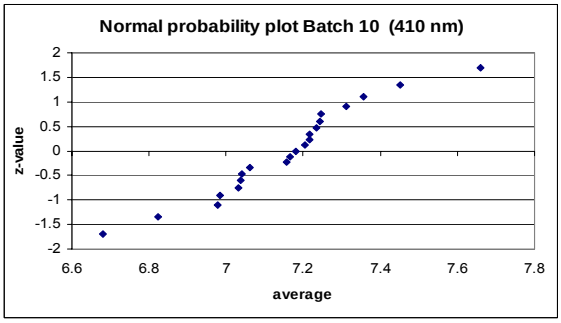
Shown are laboratory averages and standard deviations of all laboratories.

Normal probability plot: only accepted sets of results are shown

Batch 10, 410 nm (discarded results shaded)

lab #	result 1	result 2	result 3	result 4
lab1	3.678[1]	3.962[1]	4.634[1]	5.448[1]
lab2	7.150	7.232	7.337	7.263
lab4	7.182	7.150	7.261	7.270
lab5	7.001	6.983	7.092	7.090
lab6	6.669	6.686	6.680	6.690
lab7	7.056	7.026	7.048	7.020
lab8	7.192	7.220	7.219	7.230
lab9	7.170	7.196	7.148	7.119
lab10	6.944	6.932	7.143	7.111
lab11	7.107	7.193	7.275	7.237
lab12	7.430	7.401	7.488	7.493
lab13	7.299	7.182	7.253	7.200
lab14	7.498	7.290	7.185	6.819
lab15	6.977[2]	6.953[2]	7.005[2]	6.980[2]
lab16	7.673		7.646	
lab17	6.768	6.822	6.814	6.886
lab18	7.192	6.952	7.175	6.924
lab19	7.170	7.093	7.237	7.164
lab20	7.174	7.098	7.233	7.222
lab21	7.242	7.080	7.372	7.282
lab22	7.240	7.319	7.345	7.337
lab23	6.886	7.013	7.075	6.967
lab24	6.212	6.53	6.59	6.621
lab25	7.351	7.376	7.229	7.473



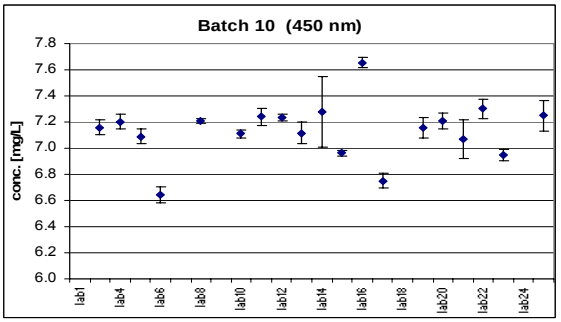


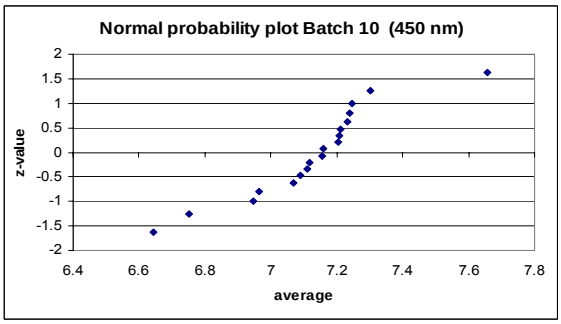
1) A repeat analysis of the 10.16 mg/L standard gave a result of 7.3, thus casting doubts on that result. The in-house method gave 6.829 mg/L

2) Many disturbing peaks that make evaluation difficult

Batch 10, 450 nm (discarded results shaded)

lab #	result 1	result 2	result 3	result 4
lab1	3.707[1]	3.912[1]	4.684[1]	5.429[1]
lab2	7.113	7.105	7.209	7.207
lab4	7.151	7.155	7.258	7.251
lab5	7.064	7.023	7.146	7.128
lab6	6.565	6.665	6.641	6.706
lab7	no results submitted			
lab8	7.192	7.202	7.215	7.236
lab9	no results submitted			
lab10	7.08	7.136	7.133	7.091
lab11	7.217	7.208	7.197	7.334
lab12	7.237	7.199	7.232	7.262
lab13	7.235	7.113	7.052	7.067
lab14	7.562	7.320	7.312	6.911
lab15	6.954[2]	6.940[2]	6.992[2]	6.965[2]
lab16	7.684		7.628	
lab17	6.802	6.789	6.723	6.689
lab18	no results submitted			
lab19	7.076	7.192	7.106	7.242
lab20	7.240	7.114	7.25	7.233
lab21	7.139	6.904	7.24	6.991
lab22	7.212	7.302	7.301	7.393
lab23	6.881	6.981	6.957	6.967
lab24	no results submitted			
lab25	7.298	7.079	7.266	7.349



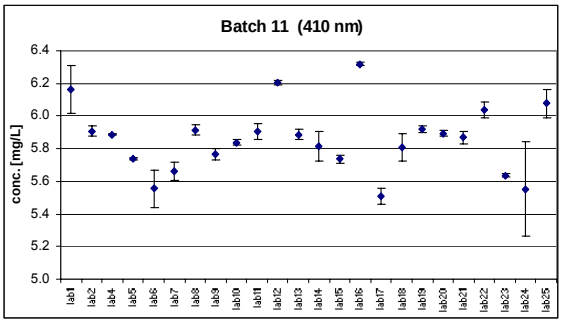


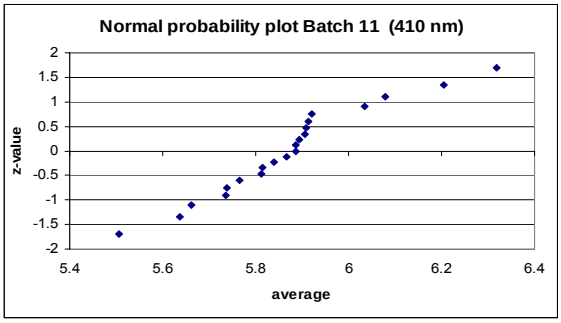
Shown are laboratory averages and standard deviations of all laboratories.

Normal probability plot: only accepted sets of results are shown

Batch 11, 410 nm (discarded results shaded)

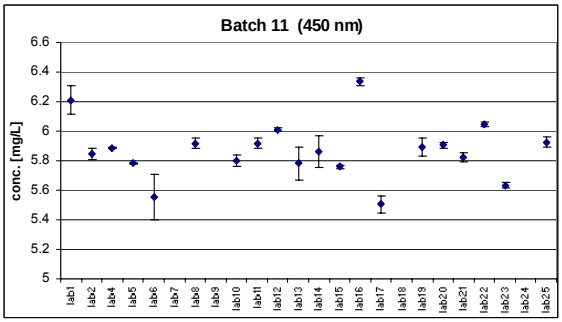
lab #	result 1	result 2	result 3	result 4
lab1	5.969	6.13	6.283	6.263
lab2	5.905	5.947	5.909	5.872
lab4	5.889	5.881	5.892	5.886
lab5	5.727	5.738	5.749	5.743
lab6	5.478	5.5135	5.504	5.726
lab7	5.617	5.616	5.724	5.686
lab8	5.894	5.879	5.945	5.939
lab9	5.819	5.743	5.755	5.749
lab10	5.837	5.841	5.818	5.861
lab11	5.857	5.882	5.966	5.925
lab12	6.219	6.207	6.191	6.206
lab13	5.866	5.932	5.886	5.861
lab14	5.946	5.759	5.775	5.780
lab15	5.737	5.711	5.767	5.731
lab16	6.311		6.328	
lab17	5.574	5.473	5.516	5.465
lab18	5.899	5.821	5.827	5.698
lab19	5.936	5.903	5.944	5.903
lab20	5.919	5.879	5.887	5.891
lab21	5.839	5.882	5.915	5.835
lab22	6.023	5.987	6.102	6.031
lab23	5.643	5.620	5.645	5.639
lab24	5.979	5.360	5.485	5.382
lab25	5.976	6.130	6.038	6.168

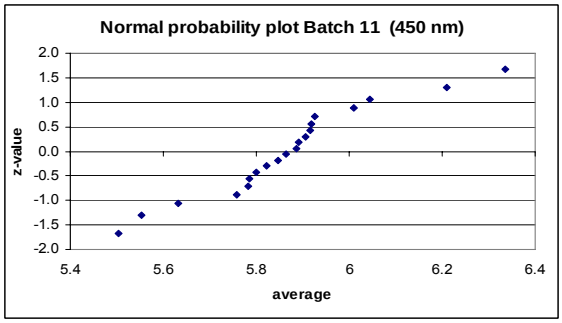




Batch 11, 450 nm (discarded reslts shaded)

lab #	result 1	result 2	result 3	result 4
lab1	6.068	6.268	6.264	6.239
lab2	5.815	5.894	5.855	5.824
lab4	5.884	5.88	5.891	5.893
lab5	5.78	5.783	5.784	5.790
lab6	5.490	5.547	5.406	5.769
lab7	no results submitted			
lab8	5.884	5.892	5.953	5.937
lab9	no results submitted			
lab10	5.8	5.766	5.781	5.853
lab11	5.962	5.899	5.929	5.884
lab12	5.996	6.013	6.016	6.017
lab13	5.750	5.941	5.752	5.687
lab14	6.01	5.755	5.83	5.857
lab15	5.776	5.752	5.76	5.748
lab16	6.318		6.352	
lab17	5.538	5.554	5.500	5.424
lab18	no results submitted			
lab19	5.947	5.949	5.823	5.85
lab20	5.934	5.887	5.902	5.899
lab21	5.821	5.855	5.83	5.784
lab22	6.040	6.038	6.034	6.065
lab23	5.642	5.605	5.636	5.647
lab24	no results submitted			
lab25	5.966	5.927	5.923	5.887



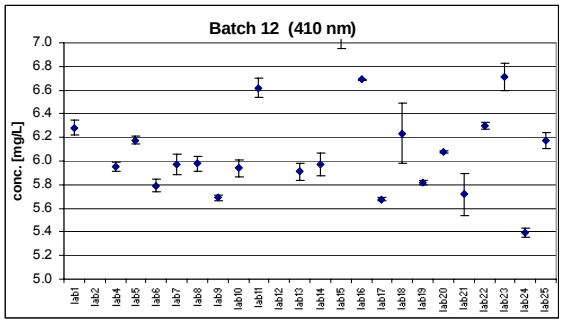


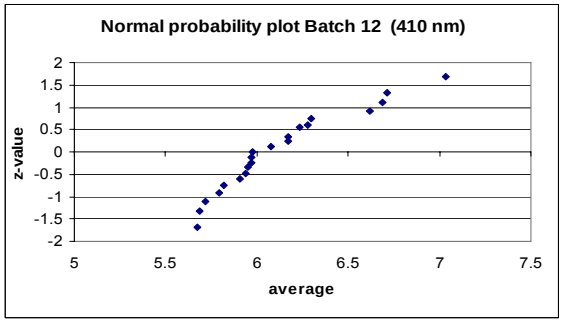
Shown are laboratory averages and standard deviations of all laboratories.

Normal probability plot: only accepted sets of results are shown

Batch 12, 410 nm (discarded results shaded)

ab #	result 1	result 2	result 3	result 4
lab1	6.372	6.241	6.247	6.259
lab2	14.711	14.739	14.863	14.786
lab4	6.010	5.942	5.944	5.912
lab5	6.147	6.221	6.156	6.171
lab6	5.753[2]	5.741[2]	5.852[2]	5.824[2]
lab7	5.879	5.948	5.979	6.079
lab8	6.065	5.914	5.968	5.964
lab9	5.717	5.690	5.691	5.657
lab10	6.046	5.919	5.906	5.880
lab11	6.721	6.531	6.573	6.650
lab12	9.587	9.576	9.525	9.483
lab13	5.813	5.894	5.963	5.964
lab14	6.078	5.886	6.020	5.894
lab15	6.979[2]	7.148[2]	7.034[2]	6.976[2]
lab16	6.685		6.694	
lab17	5.672	5.657	5.694	5.679
lab18	6.320	6.379	6.377	5.855
lab19	5.836	5.805	5.839	5.795
lab20	6.072[1]	6.071[1]	6.094[1]	6.079[1]
lab21	5.561	5.905	5.561	5.839
lab22	6.292	6.260	6.320	6.315
lab23	6.890	6.663	6.644	6.652
lab24	5.347	5.376	5.431	5.423
lab25	6.168	6.256	6.098	6.162



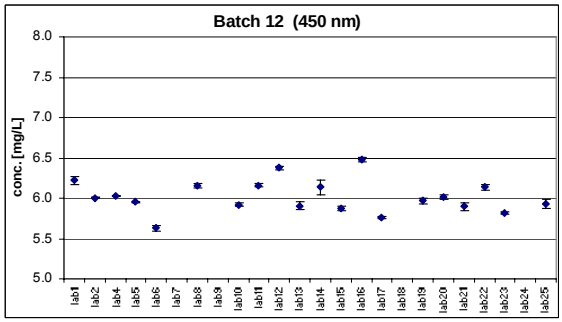


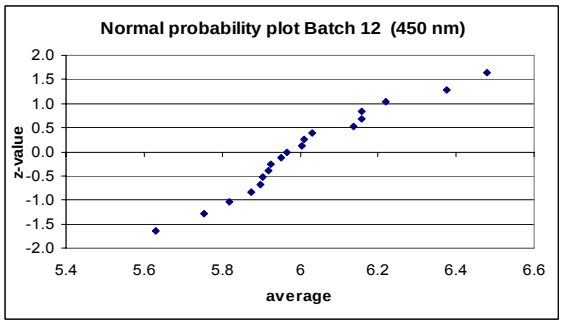
1) less interferences at 450 nm than at 410 nm

2) The samples contain many other components that seem to stay very long in the system.

Batch 12, 450 nm (discarded results shaded)

lab #	result 1	result 2	result 3	result 4
lab1	6.27	6.221	6.156	6.236
lab2	5.999	6.000	6.001	6.011
lab4	6.036	6.028	6.03	6.029
lab5	5.937	5.961	5.96	5.948
lab6	5.613	5.671	5.586	5.648
lab7	no results submitted			
lab8	6.187	6.129	6.171	6.141
lab9	no results submitted			
lab10	5.940	5.899	5.899	5.94
lab11	6.133	6.158	6.158	6.181
lab12	6.382	6.402	6.355	6.368
lab13	5.920	5.920	5.834	5.944
lab14	6.229	6.076	6.201	6.040
lab15	5.891	5.827	5.89	5.885
lab16	6.501		6.459	
lab17	5.753	5.766	5.734	5.768
lab18	no results submitted			
lab19	5.941	5.992	6.004	5.932
lab20	6.046	5.983	6.008	6.003
lab21	5.887	5.854	5.968	5.884
lab22	6.126	6.097	6.178	6.147
lab23	5.840	5.810	5.805	5.816
lab24	no results submitted			
lab25	5.872	5.993	5.956	5.881





Shown are laboratory averages and standard deviations of all laboratories.

Normal probability plot: only accepted sets of results are shown

Sequence of outlier elimination

Outliers at 410 nm

Batch 2
- Lab 24, Cochran outlier
- Lab 21, Cochran outlier
- Lab 25, Cochran outlier

Batch 3
- Lab 24, Cochran and Hawkins outlier
- Lab 21, Hawkins outlier
- Lab 11, 18, 9: Cochran outliers (kept)

Batch 4
- Lab 24, Cochran and Hawkins outlier
- Lab 15, 13, 18, 9: Cochran outliers (kept)

Batch 5
- Lab 5, Cochran outlier
- Lab 24, Cochran outlier
- Lab 18, Cochran outlier

Batch 6
- Lab 4, Cochran outlier
- Lab 25, 14, 21, 18 Cochran outliers (kept)

Batch 7
- Lab 1, Cochran and Hawkins outlier
- Lab 17, Cochran outlier

Batch 9
- Lab 24, Cochran outlier
- Lab 1, Cochran and Hawkins outlier
- Lab 9 and 15 eliminated due to normality plot

Batch 10
- Lab 1, Cochran and Hawkins outlier
- Lab 14, Cochran outlier
- Lab 24, Cochran outlier

Batch 11
- Lab 24, Cochran outlier
- Lab 1, Cochran outlier
- Lab 6, Cochran outlier

Batch 12
- Lab 2, Hawkins outlier
- Lab 12, Hawkins outlier
- Lab 18, 21, Cochran outliers (kept)

Outliers at 450 nm

Batch 2
- Lab 25, Cochran outlier

Batch 3
- Lab 11, Cochran outlier
- Lab 21, Hawkins outlier
- Lab 17, Cochran outlier

No more outliers, but not normal distributed.

Batch 4
- Lab 15, Cochran outlier
- Lab 13, Cochran outlier
- Lab 21, Hawkins outlier
- Lab 11, 1, 8, 5 , Cochran outlier (kept)

Batch 5
- Lab 13, Cochran outlier
- Lab 11, Cochran outlier

Batch 6
- Lab 21, Cochran outlier
- Lab 17, Cochran outlier

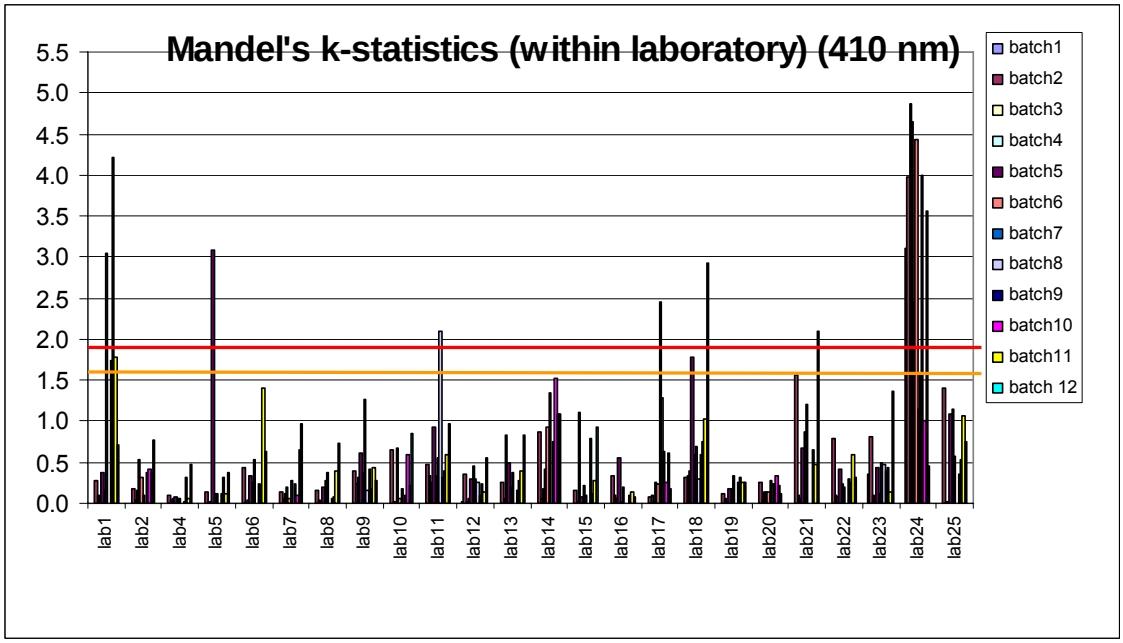
Batch 7
- Lab 1, Cochran and Hawkins outlier
- Lab 21, 14, 14, Cochran outliers (kept)

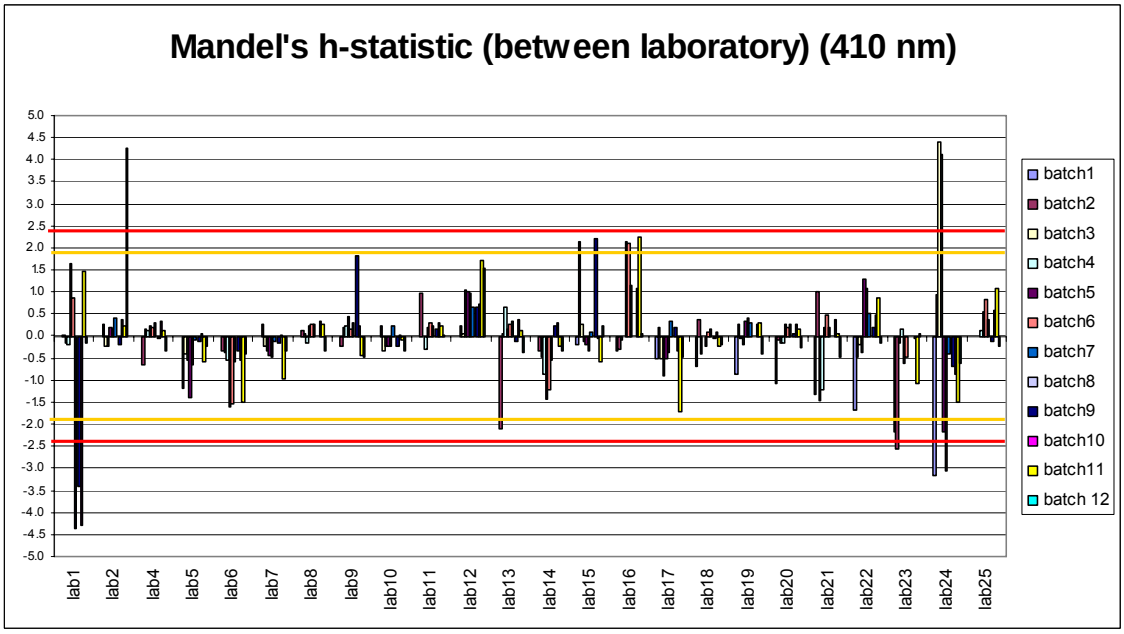
Batch 9
- Lab 15, Hawkins outlier
- Lab 1, Cochran and Hawkins outlier
- Lab 14, 17, 25, 23, 22, Cochran outliers (kept)
- Lab 2 eliminated due to normality plot

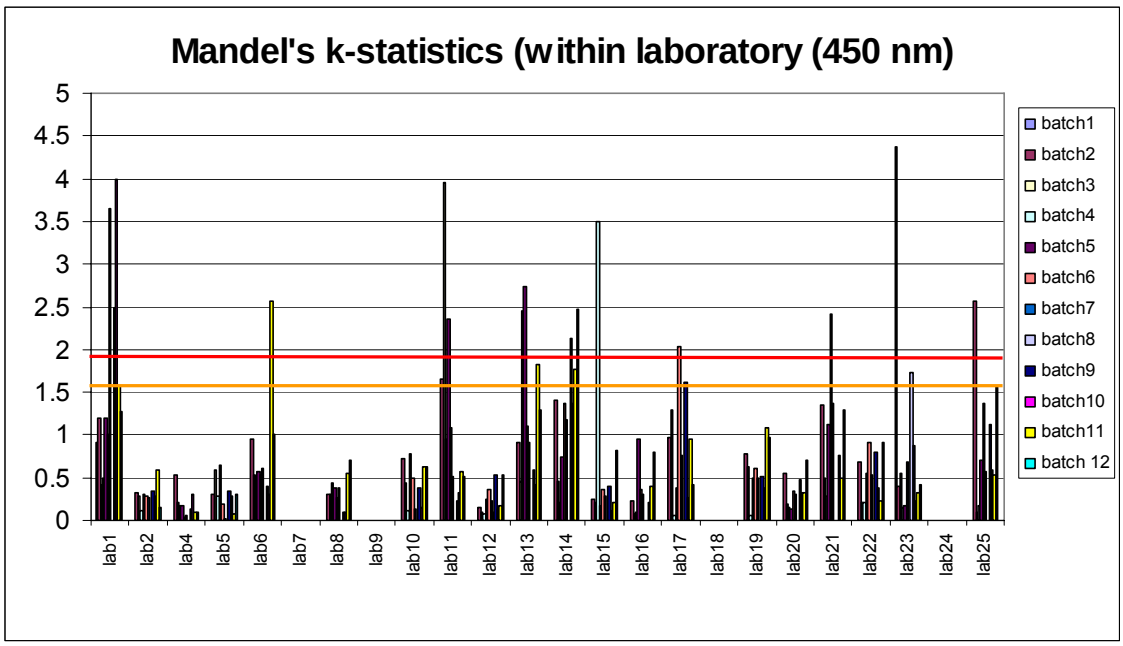
Batch 10
- Lab 1, Cochran and Hawkins outlier
- Lab 14, Cochran outlier
- Lab 21, 25, Cochran outlier (kept)

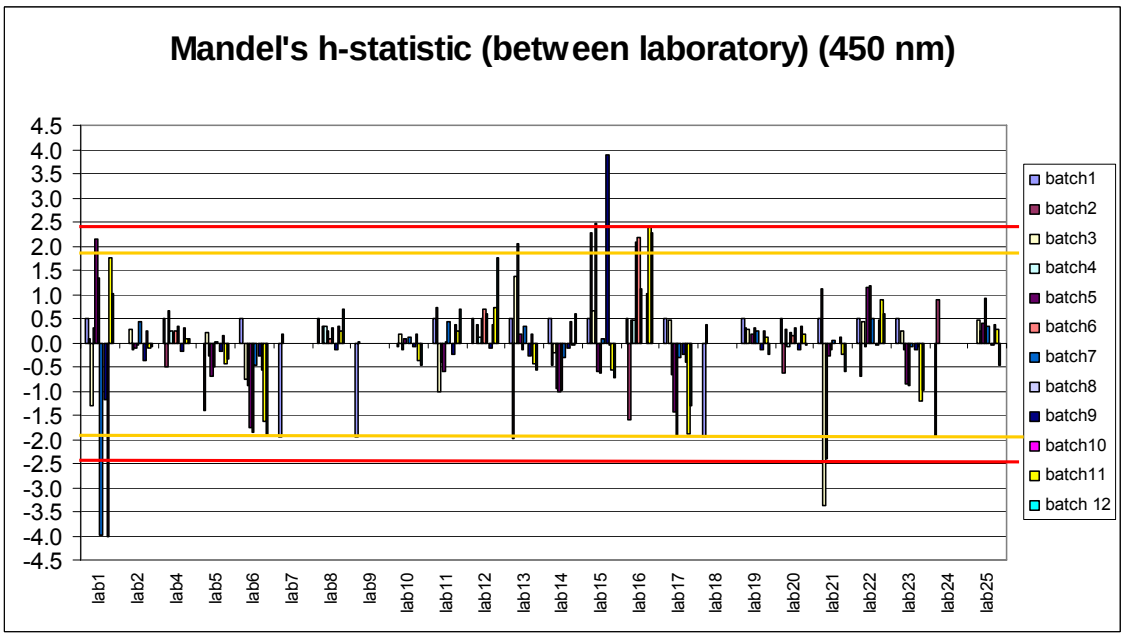
Batch 11
- Lab 6, 13, 14, 1, 19, 17 , Cochran outliers (kept)

Batch 12
- Lab 14, Cochran outlier


Mandel's k-statistics (within laboratory) (410 nm)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
lab1 lab2 lab4 lab5 lab6 lab7 lab8 lab9 lab10 lab11 lab12 lab13 lab14 lab15 lab16 lab17 lab18 lab19 lab20 lab21 lab22 lab23 lab24 lab25
batch1
batch2
batch3
batch4
batch5
batch6
batch7
batch8
batch9
batch10
batch11
batch 12


Mandel's h-statistic (between laboratory) (410 nm)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
-4.5
-5.0
lab1 lab2 lab4 lab5 lab6 lab7 lab8 lab9 lab10 lab11 lab12 lab13 lab14 lab15 lab16 lab17 lab18 lab19 lab20 lab21 lab22 lab23 lab24 lab25
batch1
batch2
batch3
batch4
batch5
batch6
batch7
batch8
batch9
batch10
batch11
batch 12


Mandel's k-statistics (within laboratory (450 nm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
lab1 lab2 lab4 lab5 lab6 lab7 lab8 lab9 lab10 lab11 lab12 lab13 lab14 lab15 lab16 lab17 lab18 lab19 lab20 lab21 lab22 lab23 lab24 lab25
batch1
batch2
batch3
batch4
batch5
batch6
batch7
batch8
batch9
batch10
batch11
batch 12

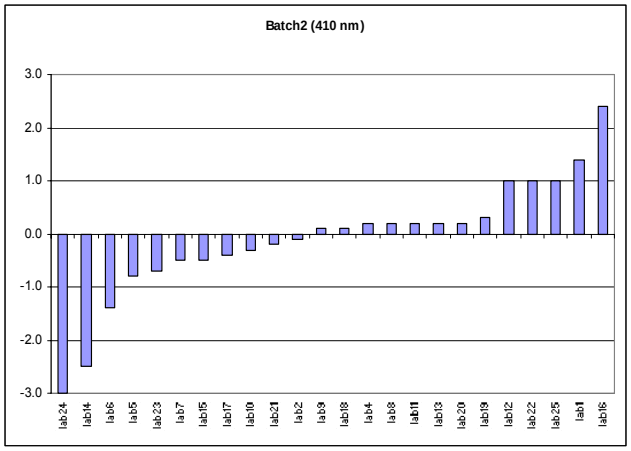

Mandel's h-statistic (between laboratory) (450 nm)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
-4.5
lab1 lab2 lab4 lab5 lab6 lab7 lab8 lab9 lab10 lab11 lab12 lab13 lab14 lab15 lab16 lab17 lab18 lab19 lab20 lab21 lab22 lab23 lab24 lab25
batch1
batch2
batch3
batch4
batch5
batch6
batch7
batch8
batch9
batch10
batch11
batch 12

z-scores

z-scores are calculated on the basis of mean and standard deviations of the accepted mean values. No z-scores were calculated for batch 1 and 8, as the target concentrations are 0 in these cases.

Results at 410 nm

	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 9	Batch 10	Batch 11	Batch 12
Lab1	1.4	-0.1	0.0	1.6	0.9	-12.2	-15.0	-12.9	1.6	0.4
Lab2	-0.1	-0.3	-0.1	0.0	0.0	0.5	-0.9	0.4	0.2	22.7
Lab4	0.2	1.3	0.6	0.0	0.1	0.2	-0.2	0.3	0.1	-0.5
Lab5	-0.8	-1.3	-0.7	-1.7	-1.0	-0.8	-0.6	-0.5	-0.7	0.1
Lab6	-1.4	-1.1	-0.7	-2.0	-2.1	-2.1	-1.5	-2.3	-1.7	-0.9
Lab7	-0.5	-0.4	-0.3	-0.7	-0.8	-0.9	-0.6	-0.6	-1.2	-0.4
Lab8	0.2	0.8	0.1	0.0	0.2	0.1	0.0	0.3	0.2	-0.4
Lab9	0.1	1.4	0.8	0.3	0.0	0.3	7.9	0.0	-0.6	-1.2
Lab10	-0.3	-0.9	-0.1	-0.5	-0.4	0.1	-1.0	-0.6	-0.2	-0.5
Lab11	0.2	0.5	-0.3	0.0	0.2	0.1	0.7	0.2	0.2	1.3
Lab12	1.0	0.9	2.5	0.9	1.1	1.2	2.8	1.4	1.8	9.0
Lab13	0.2	0.9	1.7	0.0	0.2	0.4	-0.5	0.4	0.1	-0.6
Lab14	-2.5	-1.5	-1.4	-1.8	-1.7	-2.0	1.0	0.2	-0.3	-0.4
Lab15	-0.5	1.8	0.1	-0.4	-0.6	-0.3	9.6	-0.8	-0.7	2.4
Lab16	2.4	0.3	0.3	2.2	2.5	2.5	-24.1	2.4	2.4	1.5
Lab17	-0.4	-1.7	-1.5	-0.8	-0.6	0.3	0.8	-1.6	-2.0	-1.2
Lab18	0.1	-1.2	0.3	-0.5	0.0	-0.1	-0.2	-0.5	-0.3	0.3
Lab19	0.3	0.4	0.0	0.2	0.3	0.2	-0.2	0.0	0.3	-0.8
Lab20	0.2	0.0	0.0	0.1	0.1	0.1	0.2	0.1	0.1	-0.1
Lab21	-0.2	-5.8	-2.1	0.0	0.4	0.0	-24.1	0.4	0.0	-1.1
Lab22	1.0	-0.2	-0.4	1.2	1.2	0.8	0.8	0.7	0.9	0.4
Lab23	-0.7	-0.1	0.7	-0.9	-0.8	-0.6	-0.1	-0.8	-1.3	1.5
Lab24	-3.0	20.2	8.7	-2.6	-4.1	-1.6	-3.1	-3.2	-1.8	-1.9
Lab25	1.0	0.6	0.6	0.4	0.9	0.4	-0.6	1.0	1.1	0.1


Batch2 (410 nm)
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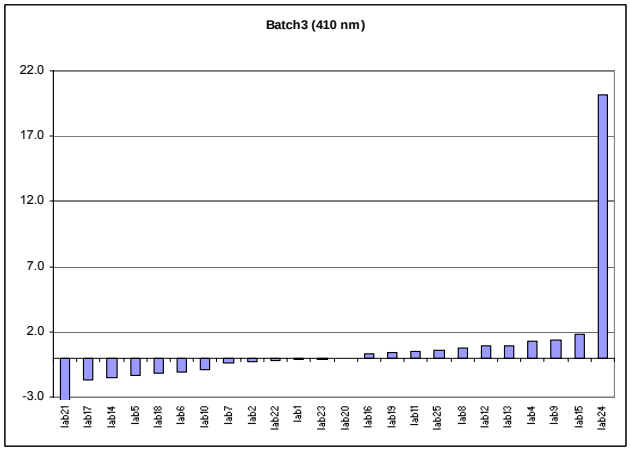
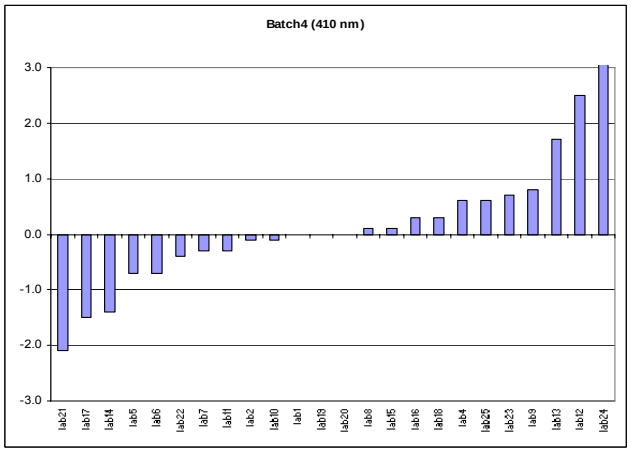
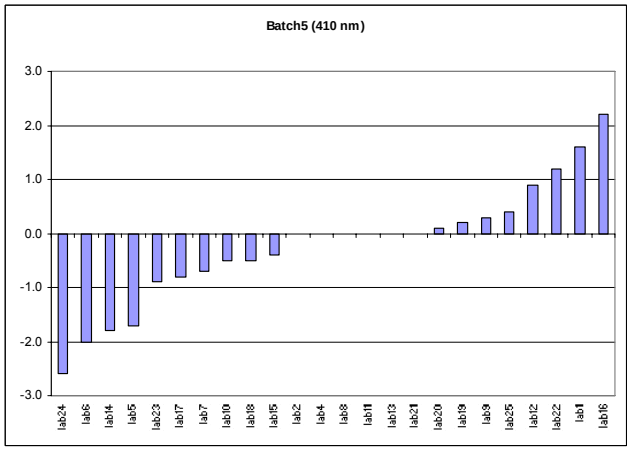
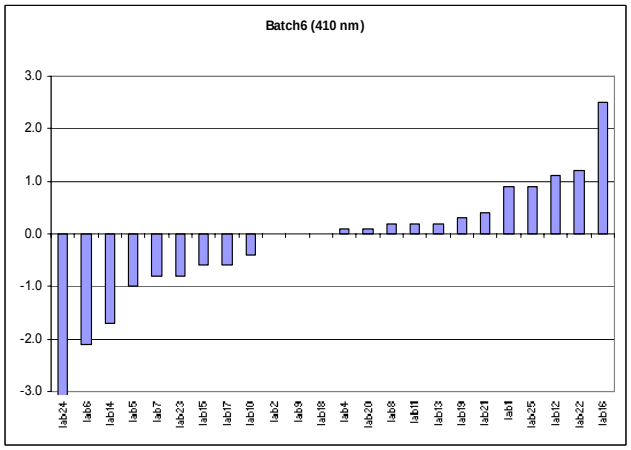
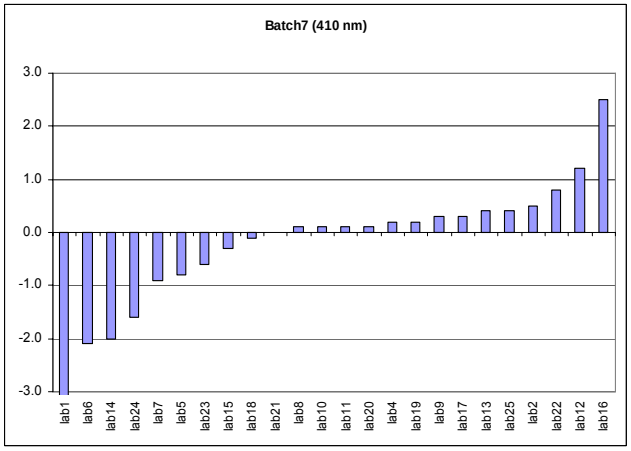
2.0
1.0
0.0
-1.0
-2.0
-3.0
lab24 lab14 lab6 lab5 lab23 lab7 lab15 lab17 lab10 lab21 lab2 lab9 lab18 lab4 lab8 lab11 lab13 lab20 lab19 lab12 lab22 lab25 lab1 lab16

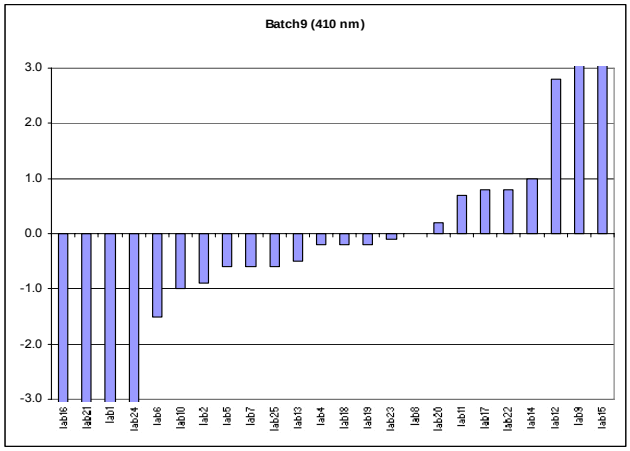

Batch3 (410 nm)
22.0
17.0
12.0
7.0
2.0
-3.0
lab21 lab17 lab14 lab5 lab18 lab6 lab10 lab7 lab2 lab22 lab1 lab23 lab20 lab16 lab19 lab11 lab25 lab8 lab12 lab13 lab4 lab9 lab15 lab24

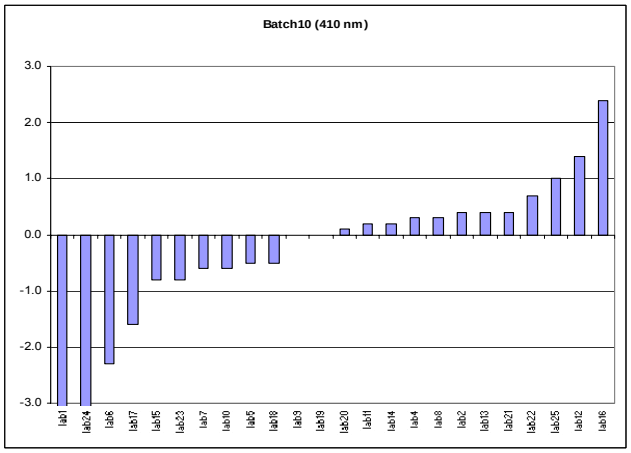

Batch4 (410 nm)
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
lab21 lab17 lab14 lab5 lab6 lab22 lab7 lab11 lab2 lab10 lab1 lab19 lab20 lab8 lab15 lab16 lab18 lab4 lab25 lab23 lab9 lab13 lab12 lab24

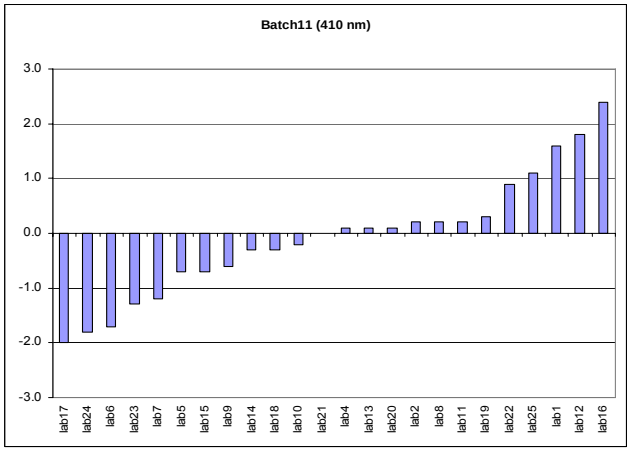

Batch5 (410 nm)
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
lab24 lab6 lab14 lab5 lab23 lab17 lab7 lab10 lab18 lab15 lab2 lab4 lab8 lab11 lab13 lab21 lab20 lab19 lab9 lab25 lab12 lab22 lab1 lab16

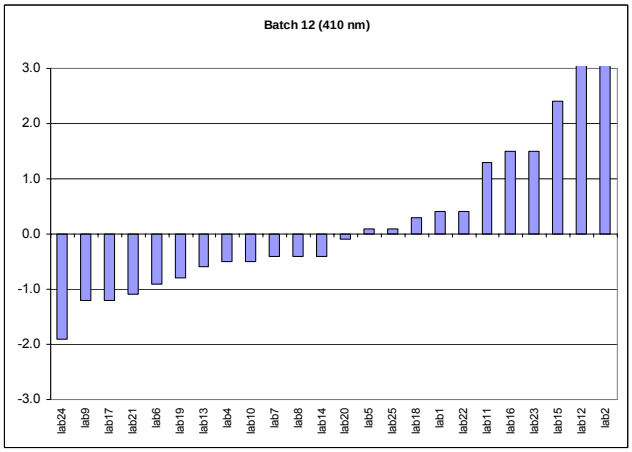

Batch6 (410 nm)
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
lab24 lab6 lab14 lab5 lab7 lab23 lab15 lab17 lab10 lab2 lab9 lab18 lab4 lab20 lab8 lab11 lab13 lab9 lab21 lab1 lab25 lab12 lab22 lab16


Batch7 (410 nm)
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
lab1 lab6 lab14 lab24 lab7 lab5 lab23 lab15 lab18 lab21 lab8 lab10 lab11 lab20 lab4 lab19 lab9 lab17 lab13 lab25 lab2 lab22 lab12 lab16


Batch9 (410 nm)
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
lab16 lab21 lab1 lab24 lab6 lab10 lab2 lab5 lab7 lab25 lab13 lab4 lab18 lab9 lab23 lab8 lab20 lab11 lab17 lab22 lab14 lab12 lab9 lab15

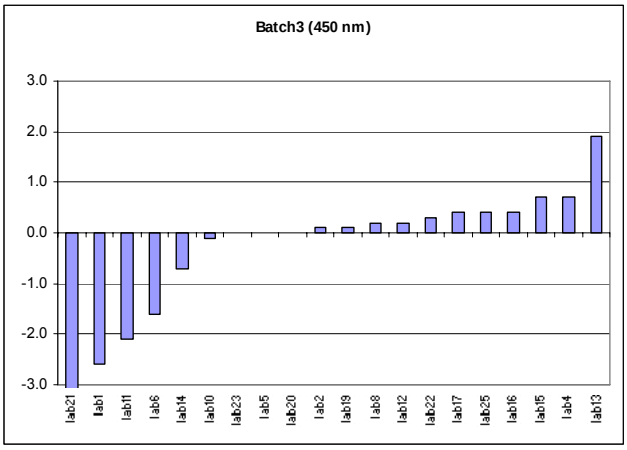

Batch10 (410 nm)
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
lab1 lab24 lab6 lab7 lab15 lab23 lab7 lab10 lab5 lab18 lab3 lab19 lab20 lab11 lab14 lab4 lab8 lab2 lab13 lab21 lab22 lab25 lab12 lab16

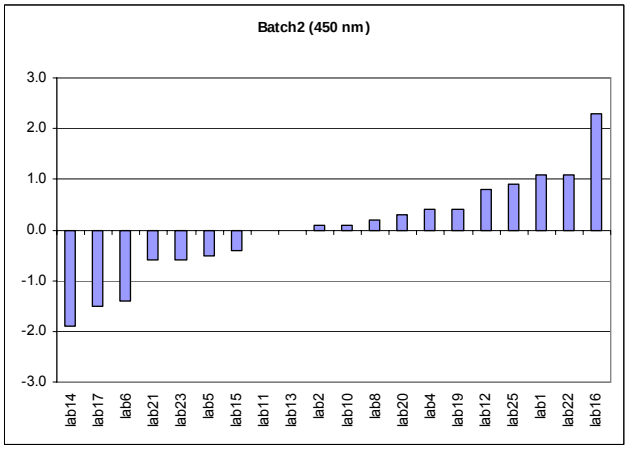

Batch11 (410 nm)
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
lab17 lab24 lab6 lab23 lab7 lab5 lab15 lab9 lab14 lab18 lab10 lab21 lab4 lab13 lab20 lab2 lab8 lab11 lab19 lab22 lab25 lab1 lab12 lab16

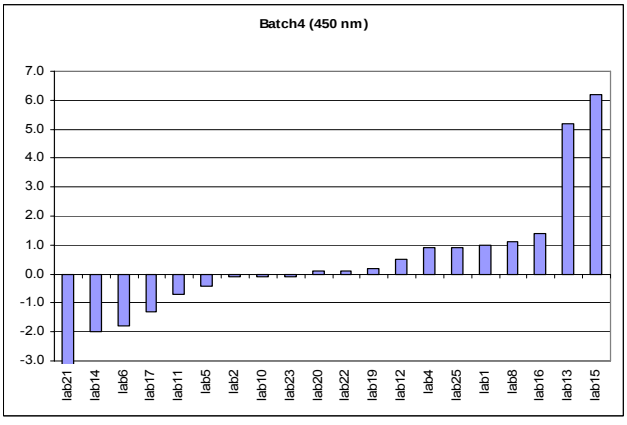

Batch 12 (410 nm)
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
lab24 lab9 lab17 lab21 lab6 lab19 lab13 lab4 lab10 lab7 lab8 lab14 lab20 lab5 lab25 lab18 lab1 lab22 lab11 lab16 lab23 lab15 lab12 lab2

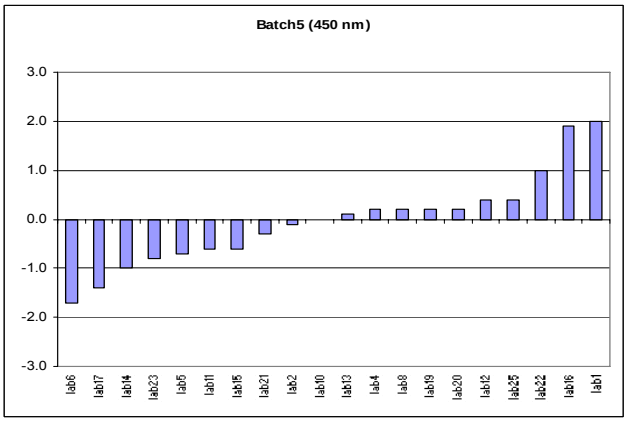
Results at 450 nm

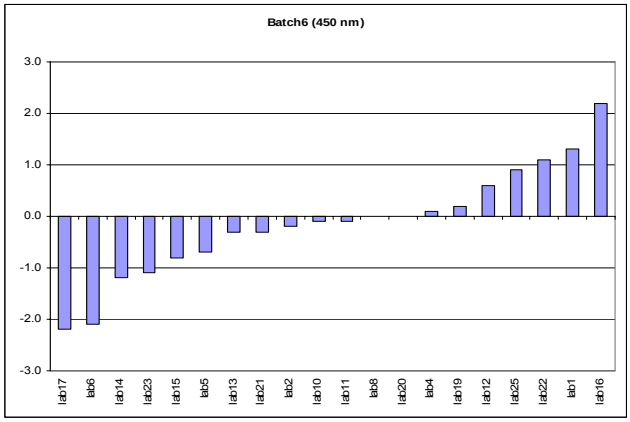
	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 9	Batch 10	Batch 11	Batch 12
Lab1	1.1	-2.6	1.0	2.0	1.3	-11.2	-14.9	-12.1	1.7	1.0
Lab2	0.1	0.1	-0.1	-0.1	-0.2	0.6	-3.2	0.1	-0.1	0.0
Lab4	0.4	0.7	0.9	0.2	0.1	0.3	-0.2	0.3	0.1	0.1
Lab5	-0.5	0.0	-0.4	-0.7	-0.7	-0.5	-0.3	-0.2	-0.4	-0.3
Lab6	-1.4	-1.6	-1.8	-1.7	-2.1	-1.9	-1.6	-2.2	-1.6	-1.8
Lab7										
Lab8	0.2	0.2	1.1	0.2	0.0	0.2	0.3	0.4	0.2	0.7
Lab9										
Lab10	0.1	-0.1	-0.1	0.0	-0.1	-0.3	1.1	-0.1	-0.3	-0.4
Lab11	0.0	-2.1	-0.7	-0.6	-0.1	0.6	-1.1	0.5	0.2	0.7
Lab12	0.8	0.2	0.5	0.4	0.6	1.0	0.4	0.5	0.7	1.7
Lab13	0.0	1.9	5.2	0.1	-0.3	0.3	-1.7	0.0	-0.4	-0.5
Lab14	-1.9	-0.7	-2.0	-1.0	-1.2	-1.4	0.8	0.7	0.0	0.6
Lab15	-0.4	0.7	6.2	-0.6	-0.8	-0.3	59.4	-0.7	-0.6	-0.7
Lab16	2.3	0.4	1.4	1.9	2.2	2.4	0.0	2.4	2.3	2.2
Lab17	-1.5	0.4	-1.3	-1.4	-2.2	-1.4	-1.4	-1.7	-1.8	-1.2
Lab18										
Lab19	0.4	0.1	0.2	0.2	0.2	0.1	0.2	0.1	0.1	-0.2
Lab20	0.3	0.0	0.1	0.2	0.0	0.3	0.2	0.4	0.2	0.0
Lab21	-0.6	-6.1	-5.5	-0.3	-0.3	-0.4	0.0	-0.3	-0.2	-0.5
Lab22	1.1	0.3	0.1	1.0	1.1	0.8	1.4	0.8	0.9	0.6
Lab23	-0.6	0.0	-0.1	-0.8	-1.1	-0.8	0.3	-0.8	-1.2	-0.9
Lab24										
Lab25	0.9	0.4	0.9	0.4	0.9	0.3	1.7	0.5	0.3	-0.4

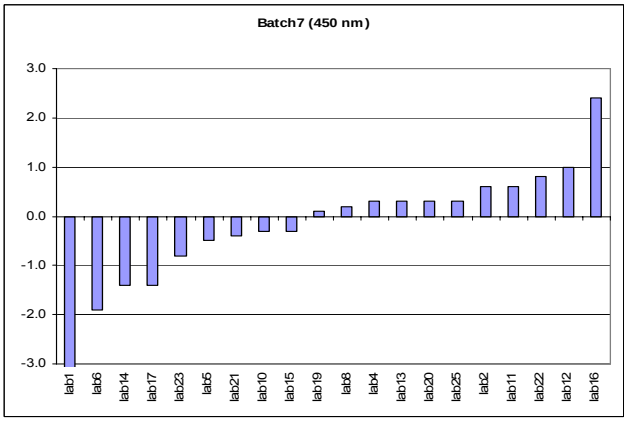


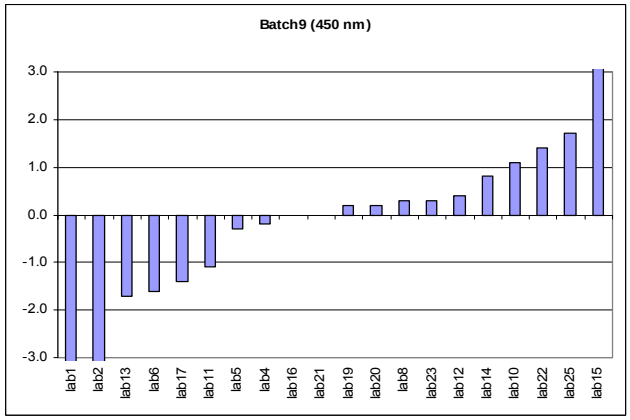


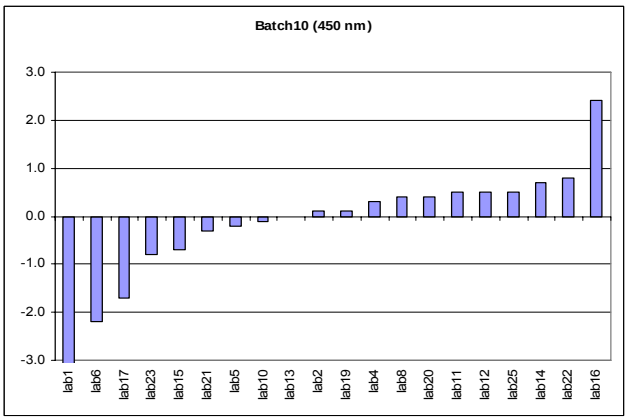





Batch6 (450 nm)
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
lab17 lab6 lab14 lab23 lab15 lab5 lab13 lab21 lab2 lab10 lab11 lab8 lab20 lab4 lab19 lab12 lab25 lab22 lab1 lab16


Batch7 (450 nm)
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
lab1 lab6 lab14 lab17 lab23 lab5 lab21 lab10 lab15 lab19 lab8 lab4 lab13 lab20 lab25 lab2 lab11 lab22 lab12 lab16


Batch9 (450 nm)
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
lab1 lab2 lab13 lab6 lab17 lab11 lab5 lab4 lab16 lab21 lab19 lab20 lab8 lab23 lab12 lab14 lab10 lab22 lab25 lab15


Batch10 (450 nm)
3.0
2.0
1.0
0.0
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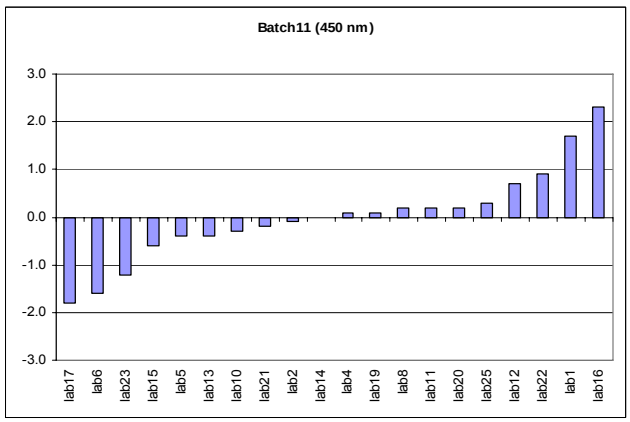
-2.0
-3.0
lab1 lab6 lab17 lab23 lab15 lab21 lab5 lab10 lab13 lab2 lab19 lab4 lab8 lab20 lab11 lab12 lab25 lab14 lab22 lab16


Batch11 (450 nm)
3.0
2.0
1.0
0.0
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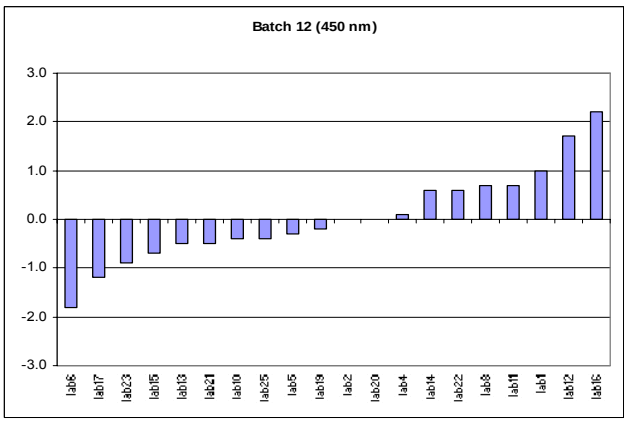
-2.0
-3.0
lab17 lab6 lab23 lab15 lab5 lab13 lab10 lab21 lab2 lab14 lab4 lab19 lab8 lab11 lab20 lab25 lab12 lab22 lab1 lab16


Batch 12 (450 nm)
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0

Community reference method of analysis of the Euromarker (solvent yellow 124)

0 Introduction/References

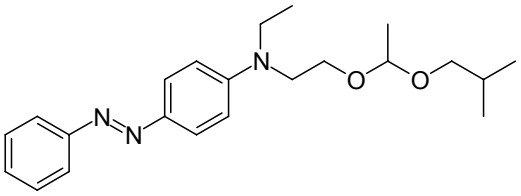
In the EC Decision 2001/574 of 13 July 2001 a common fiscal marker for gas oils and kerosene has been established.

For the proper functioning of the internal market and in particular to prevent tax evasion, Council Directive 95/60/EC of 27 November 1995 has provided for a common marking system to identify gas oils and kerosene, which are subject to a reduced excise duty rate.

The marker is:

Solvent Yellow 124 (IUPAC name: N-ethyl-N-[2-(1-isobutoxyethoxy)ethyl]-4-(phenylazo)aniline),

CAS NR: 34432-92-3



The marking level should be between 6 mg and 9 mg of marker per litre of mineral oil.

1 Scope and Field of Application

1.1 Explanation

This method describes the analysis of Solvent Yellow 124 (Sudan 455) (4.1), in the concentration range from the detection limit till 10 mg Solvent Yellow 124 per liter. When the concentration is more than 10 mg/l, a dilution with xylene (4.3) is necessary for the exact determination of the concentration. After dilution the measurement range is up to 100%.

1.2 Detection Limit

The detection limit in gas oil is to be established.

1.3 Quantification limit

The quantification limit in gas oil is to be established.

2 Definitions

*Solvent Yellow 124;*Sudan 455;

N-Ethyl-N[2-(1-isobutoxyethoxy)ethyl]-4-(fenylazo)aniline.

3 Principle and reactions

The sample is put into a sample vial. The product is analysed with normal phase chromatography and UV detection at 410 nm or 450 nm. External calibration is used.

4 Reagents and other materials

Use only reagents of acknowledged quality and MilliQ-water.

4.1 Solvent Yellow 124, purity 92 %.

The Solvent Yellow you should use for the calibration is sent to you together with the samples!!!! DO NOT use your own calibration samples!!!!

4.2 Toluene, for L.C. **Attention** 

harmful, 

Lightly inflammable.

4.3 o-Xylene p.a.. **Attention** 

Harmful.

4.4 Ethyl acetate, p.a. **Attention** 

Irritating, 

Lightly inflammable

4.5 Reference Stock solution(ca. 100 mg/l): Weigh in 55 mg Solvent Yellow (4.1) in a 500 ml volumetric flask and make up with xylene (4.3) to volume. Record the weight to four decimal places. Mix thoroughly, allow to stand for one night. Then thoroughly mix again and prepare the calibration solutions

4.6 Calbration solutions:

Concentration	Volume reference stock solution	Final volume volumetric flask
Ca. 10 mg/L	10 ml	100 ml
Ca. 5 mg/L	5 ml	100 ml
Ca. 1 mg/L	1 ml	100 ml

The volumetric flasks grade B or better.
The pipettes of 1 ml, 2 ml and 10 ml capacity, grade B or better.
Calculate the exact concentrations!!!!

4.7 Mobile phase

- Eluent Mix in a 2000 ml volumetric flask, 40 ml ethyl acetate (4.5) and 1960 ml toluene (4.2) and homogenise.

5 Apparatus

5.1 Standard laboratory glassware. Volumetric flasks and pipettes should be B-grade or better.

5.2 HPLC apparatus, equipped with the following:

5.2.1 HPLC-pump, fit for zero pulsation that delivers a constant flow at the rate required.

5.2.2 Sample injector, comprising a loop injector (manual or part of an autosampler) with a capacity of 20 µl.

5.3.3 Column, 5 µm silica length 200- 250 mm, diameter 3.0 to 5 mm ID. e.g. Waters Sperisorb 5 µm or Luna 5 Sµm Silica Phenomenex..

5.3.4 Precolumn, silica e.g. Sherisorb S5W Waters. Use is advised, but not obligatory.

5.3.5 Column oven: should be used when the retention time of the SY124 peak is not stable from run to run. Temperature 40 ^{0}C.

5.3.5 **Detector: UV 410 nm, or diode array both 410 nm and 450 nm**.

5.3.6 Integration system, comprising an electronic integrator with calculating and reporting capabilities, compatible with the output of the detector.

6 Procedure

6.1 General

take a representative sample of the product to be analysed.

6.2 Pre-treatment of the sample

Transfer sample to sample vial. Filter the sample when it is dirty through a syringe filter e.g. 0.45 µm PTFE.

6.3 Determination

Before analyzing the samples first the stability of the HPLC system and the retention time of the SY124 have to be checked. Inject the calibration solution of 10 mg/L three times. The relative standard deviation of the peak area of the 3 injections should be less then 1 %. The retention time of SY124 has to be between 2 and 4 times the retention time of the void volume (t0) and the relative standard deviation of the retention time should be less then 2 %. If the retention time is too fast or too slow the eluent has to be adapted. The addition of ethyl acetate to the eluent results in a shorter retention time.

When the system check is passed:

Both the samples and the calibration vials have to be analyzed in duplicate.

Start with the 3 samples of the calibration. Next 12 samples are analyzed. Then the calibration is repeated. Next the other 12 samples are analyzed and the sequence is ended wih the 3 calibration

samples. So the sequence is ended with a calibration. The calibration curve is forced through zero. When the correlation coefficient of the linear regression of all the calibration points (the total of calibration points will be 18) is more then 0.99 the calibration is adequate. If the correlation coefficient is less then 0.99 the system performance has to be checked and if possible improved. However when improvement is not achieved results still can be reported together with the correlation coefficient.

In annex 1 the sequence for the autosampler is given.

7 Precision

7.1 Repeatibility

The difference between the results of two single determinations, carried out in rapid succession by the same operator under the same conditions on identical test material, shall not exceed the values in 95 % of the analyses, for samples with a content of:

- 0,12 g/1000 l: Under investigaion
- 6,0 g/1000 l: Under investigation

7.2 Reproducibility

The difference between the results of two single and independent determinations, obtained by two operators working indifferent laboratories under different conditions on identical material, shall not exceed in 95 % of the analyses, for samples with a content of:

- 0,12 g/1000 l: Under investigation
- 6,0 g/1000 l: Under investigation

7.3 Measurement uncertainty

The measurement uncertainty for:

- 0,12 g/1000 l is: Under investigation
- 6,0 g/1000 l is: Under investigation

8. Literature

8.1 EC Directive 95/60 of. 27 November 1995, establishing a common fiscal marker for gas oils and kerosene.

8.2 Quantitative testing of Sudan Marker 455 liquid (Solvent Yellow) in Mineral Oils by means of liquid chromatography (HPLC), Joint Research Centre Ispra 1999.

Annex 1 1/1

SY124

Date :

Sequence Number :

Initials :

Sequence :

Nr	Vial	Nr	Vial
1	Calibration 1 mg/L	24	
2	Calbration 5 mg/L	25	
3	Calibration 10 mg/L	26	
4		27	
5		28	
6		29	
7		30	
8		31	Calibration 1 mg/L
9		32	Calbration 5 mg/L
10		33	Calibration 10 mg/L
11			
12			
13			
14			
15			
16	Calibration 1 mg/L		
17	Calbration 5 mg/L		
18	Calibration 10 mg/L		
19			
20			
21			
22			
23			

Chromatographic columns used

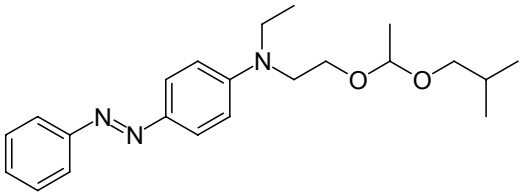
Laboratory code	columns
1	Waters Spherisorb 5um 4.6 x 250 mm
2	Spherisorb 5µm Silica 4.6x250mm
3	20 cm 5 µm Nucleosil Si 50 normal phase column 4.6 mm internal diameter
4	Phenomenex Luna 5µ Silica (2) 250 x 4,6 mm
5	Spherisorb 5µm silica 4,0x250mm analytical cartridge
6	Merck Lichrospher Si60 5 µ , 250 * 4 mm
7	Merck Lichrosorb Si 60 (5 microns length 250 mm)
8	Bischoff, Hypersil Silica 5µm, 250x4,6 mm
9	Merck Lichrocart 250-4 Spherisorb Si (5 um)
10	LiChroCART 250-4 LiChrospher Si60 5µm, Merck
11	Merck LiChroCart 250-4, Spherisorb Si 5 µm
12	Nucleosil 100-5, length:250 mm
13	Phenomenex Luna 5u Silica (2) 100A.
14	Waters Spherisorb Silica 5u, 250x4,6 mm
15	HYPERSIL Silica 250 mm, ID 4,6mm, Particle size (um) 5
16	SEPARON SIVSK 5µm 250 x 4 mm
17	Restek allure 25 cm 4.6 mcrns 5 mcrns cat.9260575
18	Waters Spherisorb 5µm Silica 4,0x250mm
19	Spherisorb w5 µm 25 x 0.46
20	Spherisorb Si 250 mm 5 µ
21	Column EC 250/4.6 NUCLEOSIL 100-5 OH
22	Spherisorb S5W –precolumn; Spherisorb 5 µm Silica 4 x 250 mm –analytical column
23	Luna 5 um silica 4.6 * 250 mm
24	S5W SPHERISORB
25	Waters Spherisorb 5um Si 4,0x250mm

Community reference method for the determination of the Euromarker (solvent yellow 124) in gas oils and kerosene.

0 Introduction/References

In the EC Decision 2001/574 of 13 July 2001 a common fiscal marker for gas oils and kerosene has been established.
For proper functioning of the internal market and in particular to prevent tax evasion, Council Directive 95/60/EC of 27 November 1995 has provided for a common marking system to identify gas oils and kerosene, which are subject to a reduced excise duty rate. This publication provides a method for the determination of SY124 in gas oil and kerosene. It shall be applied as a reference method in points of controversy for the examination of marked low tax mineral oils and mixtures with Diesel fuel.

The marker is:
Solvent Yellow 124(IUPAC name: N-ethyl-N-[2-(1-isobutoxyethoxy)ethyl]-4-(phenylazo)aniline), CAS NR: 34432-92-3



The marking level has to be between 6 mg and 9 mg of marker per litre of mineral oil.

1 Scope and Field of Application

1.1 Explanation
This method describes the determination of Solvent Yellow 124, in the concentration range from the detection limit till 10 mg Solvent Yellow 124 per liter. When the concentration is higher than 10 mg/l, a dilution with xylene (4.3) is necessary for the exact determination of the concentration.

1.2 Detection Limit
The detection limit in gas oil and kerosene is 0.02 mg/L.

1.3 Quantification limit
The quantification limit in gas oil and kerosene is 0.07 mg/L.

2 Principle and reactions

The sample is placed into a sample vial. The product is separated with normal (straight) phase chromatography and UV/VIS detection at 450 nm. Additional information can be obtained by analysing the samples with Diode Array detection employing also 410 nm. External calibration is used, the purity of the applied SY124 should be considered.

3 Reagents and other materials

Use only reagents of acknowledged quality and MilliQ-water.

3.1 Solvent Yellow 124 suppliers are John Hogg, BASF and Orgachim.

3.2 Toluene, for L.C. Attention 

harmful, 

highly flammable.

3.3 o-Xylene p.a.. Attention 

Harmful, Irritating to skin.

3.4 Ethyl acetate, p.a. Attention 

Irritating to eyes, 

highly flammable.

4 Apparatus

4.1 Standard laboratory glassware. Volumetric flasks (2000 ml and 100 ml) and pipettes (1ml, 5 ml and 10 ml) should be B-grade or better.
4.2 HPLC apparatus, equipped with the following:.
4.2.1 HPLC-pump, fit for zero pulsation that delivers a constant flow at the rate required.
4.2.2 Sample injector, comprising of a loop injector (manual or part of an autosampler) with a capacity of 20 µl.
4.2.3 Column, 5 µm silica length 200- 250 mm, diameter 3.0 to 5 mm ID. e.g. Waters Spherisorb 5 µm or Luna 5 µm Silica Phenomenex..
4.2.4 Precolumn, silica e.g. Spherisorb S5W Waters. Use is advised, but not obligatory.
4.2.5 Column oven: should be used when the retention time of the SY124 peak is not stable from run to run. Temperature 40 ^{0}C.
4.2.6 Detector: UV 450 nm, or if using diode array 410 nm and 450 nm.
4.2.7 Integration system, comprising an electronic integrator with calculating and reporting capabilities, compatible with the output of the detector.

5 Procedure

5.1 General
Take a representative sample of the product to be analysed.

5.2 Pre-treatment of the sample
Transfer sample to sample vial. Filter the sample when it is dirty through a syringe filter e.g. 0.45 µm PTFE.

5.3 Mobile phase
Eluent Mix in a 2000 ml volumetric flask, 40 ml ethyl acetate (3.4) and 1960 ml toluene (3.2) and homogenise.

5.4 Reference Stock solution:
Make a reference stock solution of SY124 of 100 mg/L by weighing the amount of Solvent Yellow (3.1) needed in a 500 ml volumetric flask and by making up with xylene (3.3) to volume at a temperature of 20 ± 1°C. Record the weight to four decimal places. The purity of the applied SY124 should be considered. Mix thoroughly, allow to stand for one night. Then thoroughly mix again and prepare the calibration solutions.

5.5 Calibration solutions:

Concentration	Volume reference stock solution	Final volume volumetric flask
Approximately 10 mg/L	10 ml	100 ml
Approximately 5 mg/L	5 ml	100 ml
Approximately 1 mg/L	1 ml	100 ml

5.6. System Check
The stability of the HPLC system and the retention time of the SY124 have to be checked before analysing the samples. Inject the calibration solution of 10 mg/L three times and perform a chromatographic run for each injection. The relative standard deviation of the peak area of the three injections should be less then 1 %. The retention time of SY124 has to be between 2 to 4 times longer than the time of appearance for the signal observed for the void volume t_0. The relative standard deviation of the retention time of SY124 should be less then 2 %. If the retention time is too short or too long the eluent has to be adapted. Addition of ethyl acetate to the eluent results in a shorter retention time.

5.7 Determination.
Samples and the calibrants are analysed in duplicate.
Commence with the three calibration solutions. Maximum twelve samples can be measured in duplicate before a new calibration has to be performed. The sequence is always finalised with three calibration solutions. The calibration curve is forced through zero. If the correlation coefficient of the linear regression of all the calibration points is better then 0.999 the calibration is adequate. If the correlation coefficient is lower then 0.999 the system performance has to be checked and if possible improved.

6 <u>Precision</u>

6.1 Repeatability
The difference between the results of two single determinations, carried out in rapid succession by the same operator under the same conditions on identical test material, shall not exceed the values in 95 % of the analyses, for samples with a content of:

Range	Repeatability
0.12 –0.27 mg/L	0.03 mg/L
4-10 mg/L	0.16 mg/L

6.2 Reproducibility

The difference between the results of two single and independent determinations, obtained by two operators working indifferent laboratories under different conditions on identical material, shall not exceed in 95 % of the analyses, for samples with a content of:

Range	Reproducibility
0.12- 0.27 mg/L	0.05 mg/L
4- 10 mg/L	0.10 X

where X is the mean of the two results.

6.3 Measurement uncertainty

Measurement uncertainty can be estimated from the reproducibility data after having confirmed that one's laboratory performs equally well as those laboratories participating in the validation study. These reproducibility data do not comprise uncertainty of the calibration. This uncertainty has to be added. The uncertainty is then estimated as

$$U = k \cdot c\sqrt{u_R^2 + u_{st}^2}$$

- U expanded uncertainty
- k coverage factor (for 95 % confidence interval choose k=2)
- c concentration for which the uncertainty should be evaluated
- u_R relative uncertainty due to reproducibility
- u_{st} relative uncertainty of the the calibration standard (mainly purity). u_{st} can be ignored if it is $< 1/3\ u_R$

For c = 0.12 mg/L: u_R = 13 %
For c > 4 mg/L: u_R = 3.5 %

7. Literature

7.1 EC Directive 95/60 of. 27 November 1995, establishing a common fiscal marker for gas oils and kerosene.

7.2 Quantitative testing of Sudan Marker 455 liquid (Solvent Yellow) in Mineral Oils by means of liquid chromatography (HPLC), Joint Research Centre Ispra 1999.

7.3 Thomas Linsinger, Ger Koomen, Håkan Emteborg, Gert Roebben, Gerard Kramer, A Lamberty (2004), Validation of the Draft Community Reference Method for the determination of Solvent Yellow 124 in gas oil (Euromarker), EUR 21195 EN, ISBN 92-894-7873-X

- *Annex A*

(informative)

- *Results of interlaboratory test*

An interlaboratory test, carried out in 2004, by the European Commision, DG-JRC Institute for reference materials and Measurements and the Dutch Customs Laboratory. Report number: EUR 21195 EN, ISBN 92-894-7873-X, gave the statistical results (evaluated in accordance with ISO 5725-2) shown in Table A.1.

- Table A.1- Precision data

Sample	2	3	4	5	6	7	9	10	11	12
Number of laboratories that reported results	20	20	20	20	20	20	20	20	20	20
Number of accepted test results	19	17	17	18	18	19	15	18	20	19
Mean SY124 content mg/L	6.04	0.12	0.27	5.99	9.05	4.78	6.10	7.13	5.87	6.01
Repeatability standard deviation. s_r	0.041	0.007	0.014	0.033	0.064	0.049	0.079	0.070	0.061	0.032
Repeatability coefficient of variation. %	0.68	5.83	5.19	0.55	0.71	1.03	1.30	0.98	1.04	0.53
Repeatability limit. r ($2 \cdot \sqrt{2}$ x s_r)	0.116	0.020	0.040	0.093	0.181	0.139	0.223	0.198	0.173	0.091
Reproducibility standard deviation. s_R	0.231	0.016	0.020	0.225	0.279	0.153	0.278	0.210	0.189	0.196
Reproducibility coefficient of variation. %	3.82	13.33	7.41	3.76	3.08	3.20	4.56	2.95	3.22	3.26
Reproducibility limit. R ($2 \cdot \sqrt{2}$ x s_R)	0.653	0.045	0.057	0.636	0.789	0.433	0.786	0.594	0.535	0.554