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Comparability Study
between the LOVIBOND COD „vario" cuvette test
and the Hach™ COD cuvette test

Contents   Page

Introduction and objectives of this study

The chemical oxygen demand (COD) of water is defined as the volume-related mass of oxygen, which is equivalent to the mass of sodium dichromate digested under given operational conditions to oxidize the oxidizable compounds present in the water.

The purpose of the present study is to compare the LOVIBOND COD vario cuvette test with the corresponding Hach™ cuvette test as well as with the reference method (DIN 38409). The LOVIBOND COD vario cuvette test may be used with LOVIBOND photometers as well as with Hach* photometers. The study was sponsored by LOVIBOND.

Methods employed, reagents, and apparatus
The following LOVIBOND products were employed to perform the cuvette tests:
1) Cuvette test COD Vario 0 - 150 mg/l   (Cat. No. 420720)
2) Cuvette test COD Vario 0 - 1500 mg/l   (Cat. No. 420721)
3) Cuvette test COD Vario 0 - 15000 mg/l   (Cat. No. 420722)

The following Hach™ products have been used:
1) Cuvette test COD 0 - 150 mg/l   (No. 21258)
2) Cuvette test COD 0 - 1500 mg/l   (No. 21259)
3) Cuvette test COD 0 - 15000 mg/l (No. 24159)

A Hach™ DR/2010 Photometer was used for the photometric evaluation of both cuvette tests.

The cuvette tests were performed according to the user's manual issued by the instrument's manufacturer and the instructions covering the photometer which was used.

The standard DIN 38402 – 71 [3] was used as a reference for the comparison between methods. Because the stringent restrictions of this standard often make it difficult or impossible to determine comparability between different methods [1], Part II of this present study is based on simpler, commonly accepted comparison tests.

Sodium hydrogenphtalate solutions in water were used as standards for the analytical methods.

The actual effluent samples which were examined were of municipal and industrial effluents (ca. 50% each) taken at random upstream and downstream of treatment plants. The sewage samples were homogenized prior to analysis by an Ultra-Turrax T 50.

Part I

Proof of comparability of the Lovibond and Hach™ analytical methods for the determination of COD in effluents, to DIN 38 402-71.

I.1   Comparison of distribution, using methods capable of calibration in matrix-free solutions

Single measurements of calibration standards were used to prove the comparability of distribution. Identification of the limits of determination and quantification was performed in accordance with the calibration straight method set out in DIN 32645.

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Table 1:   Comparison of distribution (Variance F-Test);
Detection and quantification limits to DIN 32 645

$F(99\%;f_1,f_2) = \textbf{6.03}$ $f_1 = f_2 = N\text{-}2$

Measurement range COD mg/L	V_{x0}	PG	Signif. Difference	N	LOD mg/l	LOQ mg/l
0-150 Lovibond	1.46		No	10	4.2	14.2
0-150 Hach™	1.89	1.29	No	10	5.5	18.2
0-1500 Lovibond	1.15		No	10	33	113
0-1500 Hach™	0.96	1.20	No	10	28	94
0-15000 Lovibond	0.88		No	10	254	864
0-15000 Hach™	1.08	1.23	No	10	312	1055

Key :

$F(99\%;f_1,f_2)$	Table value of F-distribution
f	Number of degrees of freedom
V_{x0}	Method variation coefficient
PG	test statistic; if PG is less than F then no significant difference
N	Number of measurements
LOD	Limit of detection
LOQ	Limit of quantification

Assessment:
1. The comparison of distribution shows no significant differences between the two methods.

2. The limits of detection and quantification are comparable for both methods over all three measurement ranges tested (see part II, table 5).

The equality of both analytical methods is demonstrated with regard to distribution, limit of detection, and limit of quantification.

I.2   Proof of equality of analytical results of real samples by means of a mean value t-test.

Table 2:   Comparison of real samples by means of a mean value t-test.
Reference method: Hach™

Measurement range 0 – 150 mg/l	Sample 1		Sample 2	
	Lovibond	Hach™	Lovibond	Hach™
N	7	7	7	7
Mean value	**9.9**	**10.1**	**85**	**84**
Standard deviation	1.68	1.57	2.98	3.15
relative	17.00	15.51	3.50	3.73
Outlier	0	0	0	0
F-test-significant		No		No
MV-t-test-significant		No		No

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Measurement range 0 – 1500 mg/l	Sample 3		Sample 4	
	Lovibond	Hach™	Lovibond	Hach™
N	6	6	6	6
Mean value	**288**	**291**	**1.215**	**1.220**
Standard deviation	7.03	8.36	18.20	16.46
relative	2.44	2.87	1.50	1.35
Outlier	0	0	0	0
F-test-significant		No		No
MV-t-test-significant		No		No

Measurement range 0 – 15000 mg/l l	Sample 5		Sample 6	
	Lovibond	Hach™	Lovibond	Hach™
N	8	8	6	6
Mean value	**3293**	**3301**	**13780**	**13973**
Standard deviation	96.62	97.02	139.86	233.30
relative	2.93	2.94	1.01	1.67
Outlier	0	0	0	0
F-test-significant		No		No
MV-t-test-significant		No		No

Assessment:

1. Outlier test
   Both methods show no outliers for all six samples.

2. Test of homogeneity of variances
   All samples show no statistic significant differences of variances.

3. Mean value t-test
   Statistically significant differences in mean values were detected in none of the six samples.

The equality of the methods of Lovibond and Hach™ for real samples is demonstrated, using the mean value t-test.

I.3 Proof of the comparability of analytical results of real samples by means of orthogonal regression

Mean values of double determinations of the real samples were used in order to compare the methods by means of a orthogonal regression.

DIN 38 402 requires the analysis of at least 30 real samples, equally distributed as much as possible over the concentration range under examination. In addition, the highest and the lowest result must not deviate by more than a factor of 100. In practice it is difficult and sometimes impossible to achieve these two requirements with an acceptable degree of effort [1]. This study therefore focuses on demonstrating comparability of the two methods under examination, by restricting consideration to results greater than the limit of quantification (LOQ = 15 mg/l) up to 1500 mg/l COD.

During the test period there was a lack of samples with COD values between 4000 and 15000 mg/l.

I.3.1 Equality of the three measurement ranges

The three measurement ranges were first tested for systematic deviations by means of a orthogonal regression[1]. The results are shown in table 3. There are no hints for systematic deviations. The results for each individual measurement range are presented in the graphs 1 to 3.

Table 3 Orthogonal regression of real samples per measurement range

Measurement range 0 - 150 mg/l	Lovibond/Hach™
Slope	1.030
Intercept	-3.7
N	13
Outlier	1
prop.-syst. Deviation	No
const.-syst. Deviation	No

Measurement range 0 - 1500 mg/l	Lovibond/Hach™
Slope	0.999
Intercept	-3.7
N	25
Outlier	0
prop.-syst. Deviation	No
const.-syst. Deviation	No

Measurement range 0 - 15000 mg/l	Lovibond/Hach™
Slope	1.005
Intercept	-3.0
N	11
Outlier	1
prop.-syst. Deviation	No
const.-syst. Deviation	No

I.3.2 Comparison of methods, carrying out COD determination on real samples

The result range of 0 – 1500 mg/l COD was selected in order to prove the comparability of the two methods to DIN 38 402. This range consists of 38 data points.
Cuvettes of the measurement ranges 0 – 150 mg/l and 0 – 1500 mg/l were employed to generate the measurement values used for the calculation. The distribution of the results is shown in Figs. 1 and 2. Four data points were excluded from the calculation

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[1] This test does not exactly comply with the requirements of the DIN 38 402 due to the small number of samples in the three measurement ranges. The purpose here is merely to check whether there are any signs of deviations in the individual measurement ranges.

because the results were below the limit of quantification of approximately 15 mg/l (see table 1). The result of the comparison study using real samples in shown in table 4:

<div align="center">

Table 4   Orthogonal regression of real samples

Samples 15 – 1500 mg/l	Lovibond/Hach[™]
Slope	0.997
Intercept	-2.3
N	34
Outlier	1
const.-syst. Deviation	No
prop.-syst. Deviation	No

</div>

Graphical representation of the results from real samples
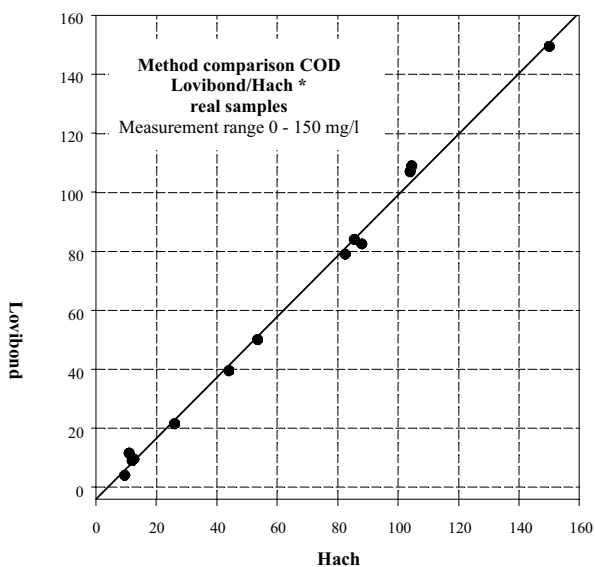
Figure 1
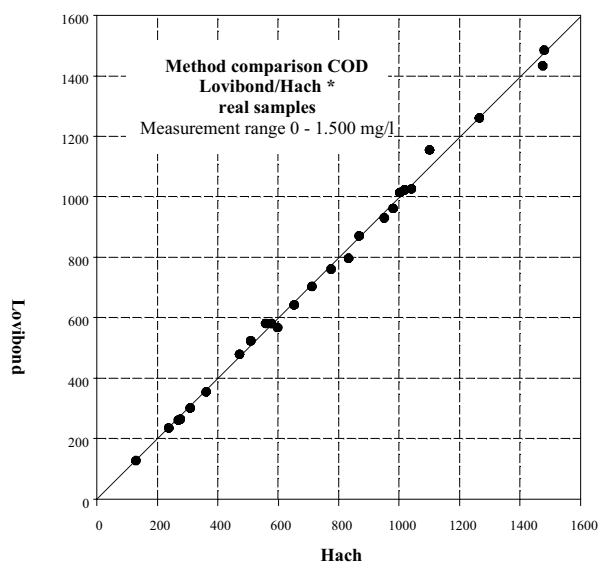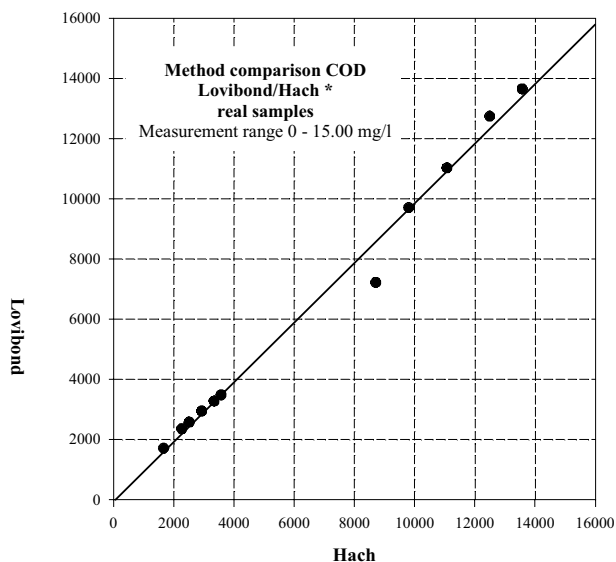Real samples 0 - 150 mg/l

Figure 2
Real samples 0 - 1500 mg/l

Method comparison COD
Lovibond/Hach *
real samples
Measurement range 0 - 150 mg/l

Method comparison COD
Lovibond/Hach *
real samples
Measurement range 0 - 1.500 mg/l

Figure 3
Real samples 0 - 15000 mg/l

Method comparison COD
Lovibond/Hach *
real samples
Measurement range 0 - 15.00 mg/l

Assessment:

1. One outlier for the data points was determined
2. The direct comparison between Lovibond/Hach™ revealed no significant proportional-systematic deviation
3. The direct comparison between Lovibond/Hach™ also revealed no significant constant-systematic deviation

The comparison between Lovibond and Hach™ demonstrates equality for both methods according to DIN 38 402-71 for values in the measurement ranges 0 - 150 and 0 - 1500 mg/l.

Comparability for values between 1500 - 15000 mg/l is highly probable but could not be demonstrated statistically due to a lack of appropriate samples (see Figure 3) .

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Graphical representation of the calibration curves

Figure 4
Calibration 0 - 150 mg/l
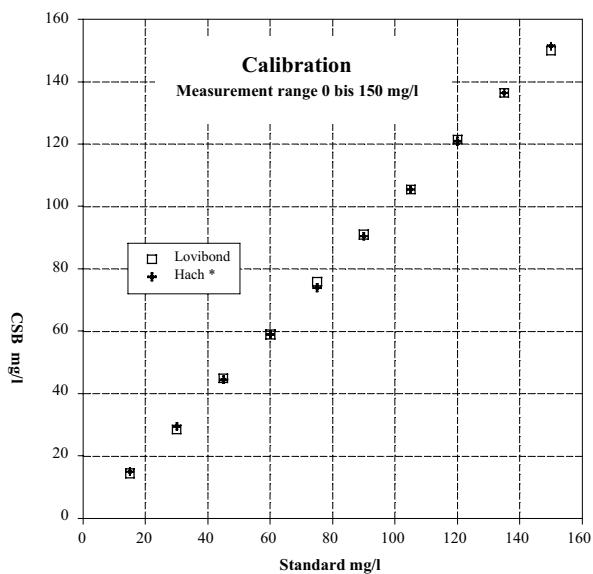
Figure 5
Calibration 0 – 1.500 mg/l

Calibration
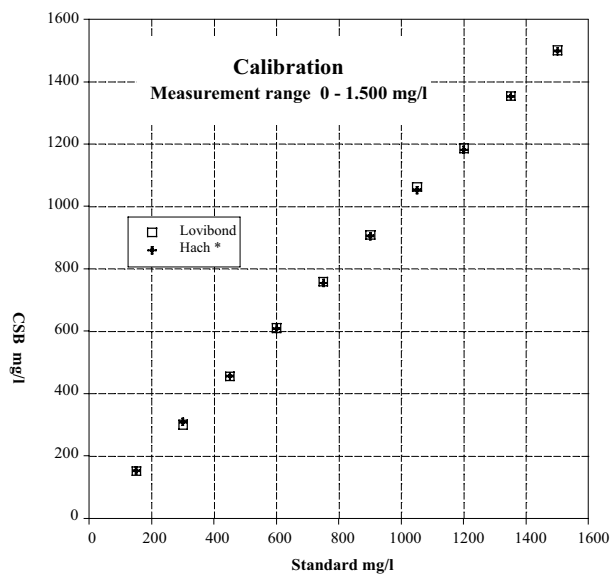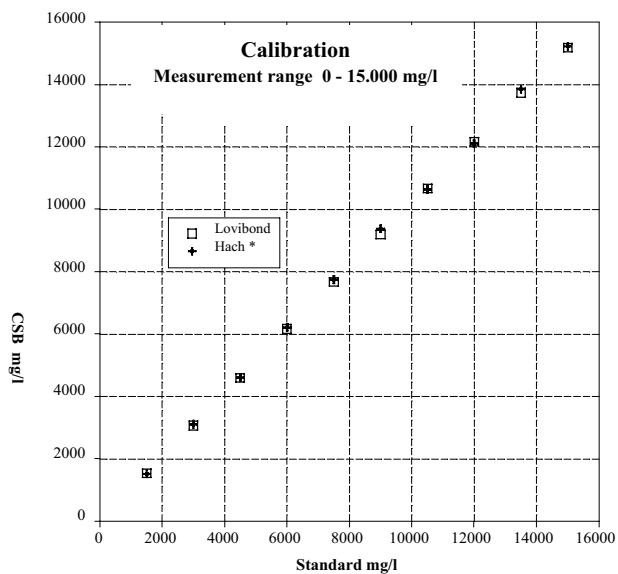**Measurement range 0 bis 150 mg/l**

Legend: □ Lovibond, + Hach *

(Y-axis: CSB mg/l, X-axis: Standard mg/l)

Calibration
Measurement range 0 - 1.500 mg/l

Legend: □ Lovibond, + Hach *

(Y-axis: CSB mg/l, X-axis: Standard mg/l)

Figure 6
Calibration 0 – 15.000 mg/l

Calibration
Measurement range 0 - 15.000 mg/l

Legend: □ Lovibond, + Hach *

(Y-axis: CSB mg/l, X-axis: Standard mg/l)

The calibrations of the methods under examination agreed extremely well for all measurement ranges.

Part II

Comparison of Lovibond and Hach™ calibrations with DIN 38409 H41

Figs. 4 to 6 show that the calibrations of the tested methods are in excellent agreement. This section deals with the comparison of the two cuvette methods using the DIN method. Table 5 shows the statistic evaluation for all three methods at the individual measurement ranges. The calculations are based on single determinations.

Table 5

Significance level P = 99%; k = 3

Method	Lovibond	Hach™	DIN
Measurement range	0 - 150 mg/l		
N	10	10	10
Intercept	-0.600	-0.933	-1.133
Slope	1.011	1.013	1.,014
Remain. std. dev.	1.22	1.58	0.71
V_{xo}	**1.46**	**1.89**	**0.63**
Correlation coeff.	0.9997	0.9995	0.9999
x_{NG}	**4..2**	**5..5**	**2.4**
x_{BG}	**14.2**	**18.2**	**8.4**
CI LOQ $_{lower}$ **)	9.6	12.3	5.7
CI LOQ $_{upper}$	27.2	34.9	16.0

**) CI LOQ = Confidence interval of the limit of quantification in accordance with DIN 32 645

Measurement range	0 – 1.500 mg/l		
N	10	10	10
Intercept	6.87	13.7	17.6
Slope	0.995	0.985	0.993
Remain. std. dev.	9.46	7.82	6.61
V_{xo}	**1.15**	**0.96**	**0.60**
Correlation coeff.	0.9998	0.9999	0.9999
x_{NG}	**33**	**28**	**23**
x_{BG}	**113**	**94**	**80**
CI LOQ $_{lower}$ **)	77	64	54
CI LOQ $_{upper}$	216	181	153

Method	Lovibond	Hach™	DIN
Measurement range	0 – 15.000 mg/l		
N	10	10	10
Intercept	127	48.0	16.7
Slope	0.999	1.016	0.989
Remain. std. dev.	72.5	90.34	50.57
V_{xo}	**0.88**	**1.08**	**0.62**
Correlation coeff.	0.9999	0.9998	0.9999
x_{NG}	**254**	**312**	**179**
x_{BG}	**864**	**1055**	**614**
CI LOQ $_{lower}$ **)	588	717	417
CI LOQ $_{upper}$	1659	2025	1178

Table 5 shows that the slopes of the calibration curves are close to the theoretical nominal value of 1 in all measurement ranges. The slope values are between 0.985 and 1.016 . The variance coefficient of the methods V_{xo} is small and has values between 0,60% and 1,89%. All correlation coefficients are higher than 0,9995.
The limits of detection and quantification are comparable. The confidence intervals (CI LOQ) overlap for the individual measurement ranges (except for measurement range 0 - 150 and 0 - 15000 mg/l for the comparison between Hach™ and DIN).

The calibrations of the methods examined are comparable with regard to distribution, sensitivity, and limits of quantification and detection.

Graphical representation of the calibration curves for the individual measurement ranges:
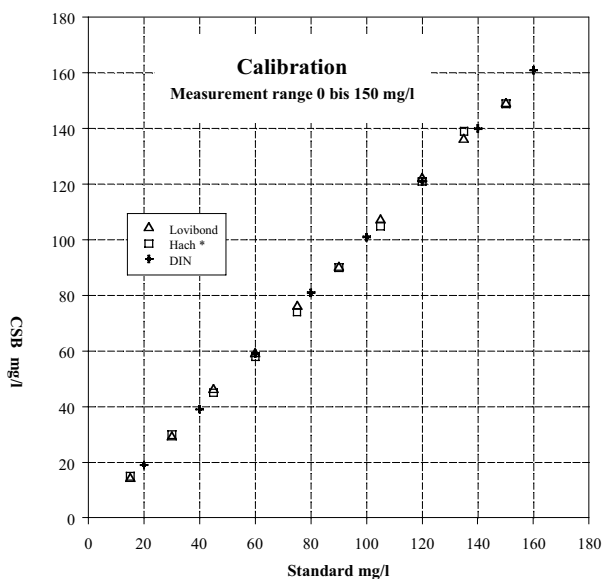
Figure 7
Measurement range 0 - 150 mg/l
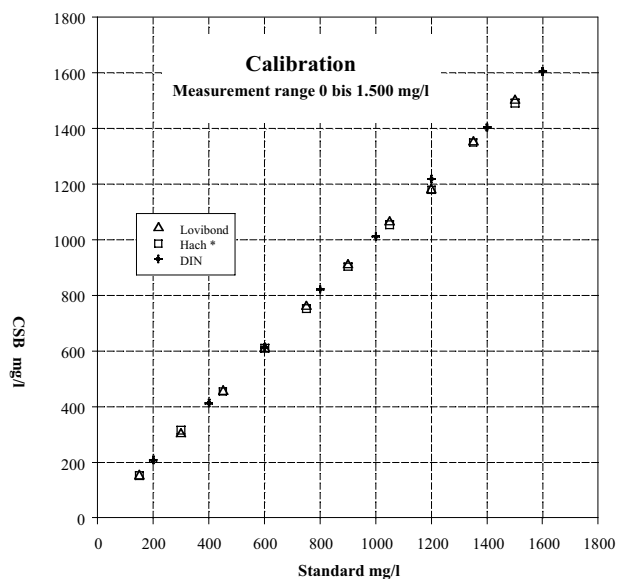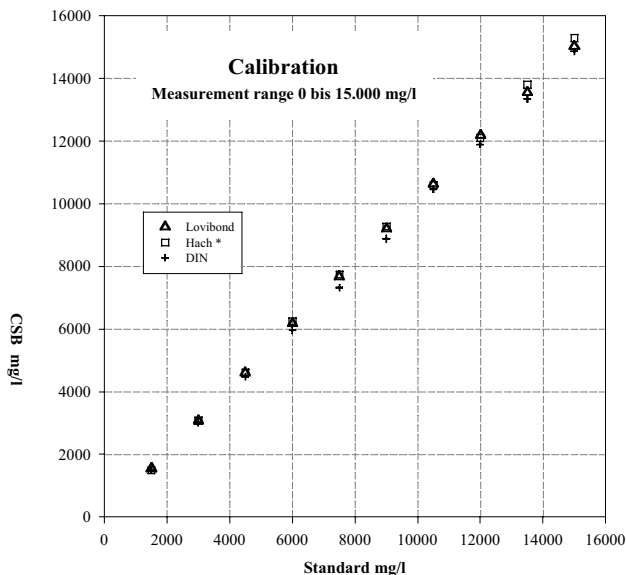
Figure 8
Measurement range 0 – 1.500 mg/l

Figure 9
Measurement range 0 – 15.000 mg/l

Literature

[1]   Furtmann, K., Lokotsch, R. : Praxisbeispiele zur Ermittlung der Gleichwertigkeit
       von Analysenmethoden, GIT Labor-Fachzeitschrift 11/2001
[2]   DIN 32 645; „Nachweis- , Erfassungs- und Bestimmungsgrenze"; Mai 1986
[3]   E DIN 38402-71; „Gleichwertigkeit von zwei Analysenverfahren aufgrund des
       Vergleiches von Analysenergebnissen und deren statistischer Auswertung;
       Vorgehensweise für quantitative Merkmale mit kontinuierlichem Wertespektrum
       (A 71)"; 2001-10

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