

Comments on the determination of Cr(VI) by test method IUC18/CEN/TS14495

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The publication of the test method CEN/TS 14495⁽¹⁾ (identical to IUC 18) last year—which focused on the determination of Cr(VI) in leather—became the subject of discussion in Germany and across the whole of Europe. The reason for this is the co-existence of two differing analytical methods for the determination of Cr(VI) in leather. Unfortunately, the European method has only reached technical specification status leaving DIN 53314⁽²⁾ as the official method of testing in Germany. The European standard is derived from the German standard and tries to overcome critical flaws in the existing procedure such as the possibility of false positives in the case of some highly coloured extracts through the introduction of a bleaching stage.

There is common agreement amongst tanners, manufacturers and users that leather should not contain any chrome (VI) at all, but if it does happen to be present, the content of Cr(VI) should be below the quantification limit of the analytical technique used. However, both standards have different quantification limits:

DIN 53314: 3 mg/kg
CEN/TS 14495: 10 mg/kg

The resulting “increase” of the quantification limit, given by the additional operating step (bleaching step) and different dilution ratios in comparison with the German standard, is confusing for the user.

The introduction of CEN/TS 14495 into the laboratory routine has shown some inconsistencies with negative effects on the efficiency, precision and sensitivity of the procedure. Vital points relating to this are presented and discussed below:

Item 1

The description of the calibration contains provisions which are critical and should be modified:

- 1.1. The minimum for calibration concentrations should be raised from 4 to 6⁽³⁾ in order to improve the statistical accuracy.
- 1.2. The blank should not be used for plotting the calibration curve.
- 1.3. The working range should be limited to the linear range of the spectrophotometer (20ml/25 already give 1.1 abs (absorbance) when using the 2cm cell).

Whilst surely most of the routine laboratories will overcome these inconveniences, in less skilled ones they might cause errors.

Item 2

Cr(VI) stock and standard solutions were made in water.

Calibrating solutions prepared starting from the standard solution were made up with buffer (*dipotassium hydrogen phosphate solution*). Over the whole working range this produced a marked difference in reaction conditions (pH). As the absorbance of the analytical solution varies with the acidity⁽⁴⁾, it is important to maintain a constant pH value.

With the given sample preparation, to make up to the mark with buffer is correct. However, especially in case of the calibrating solutions the buffer concentration should be uniform. Therefore it is advisable to prepare both stock and standard solution in buffer as well. That guarantees consistent pH values over the calibrated interval as shown in *Figure 1*.

Figure 1: Comparison of pH values of standard solutions

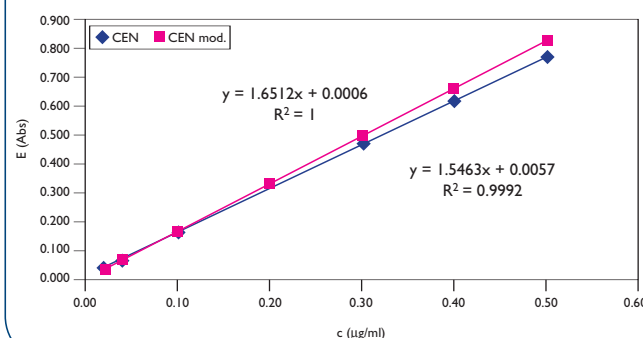
Cr(VI)(µg/ml)	pH: standard in water	pH: standard in buffer
0.08	2.05	2.07
0.2	1.98	2.07
0.4	1.72	2.06
0.6	1.53	2.07

Item 3

The use of buffer for preparing the standard solution alone does not achieve the precision expected. Improvement was attained only by changing the adding order of diphenyl carbazide (DPC) and phosphoric acid—first the phosphoric acid and then the DPC⁽⁵⁾. With this modification the sensitivity was increased (*Figure 2*) and the limit of quantification was lowered.

The calibration used in LGR investigations covered the range of 0.02 - 0.5 µg Cr(VI)/ml (cell length 2 cm). This is equivalent to a Cr(VI) content in leather of 6.25 - 156.25 ppm. ➤

Figure 2: Calibration of CEN/TS14495 and the LGR modification using a 2cm cell



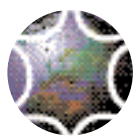


Figure 3: Precision at various Cr(VI) concentration levels (n=10) using CEN/TS 14495 and the modified procedure (LGR recommendation)

Cr(VI) µg/ml	Averages (abs)		Standard deviation (abs)		Variation coefficient %	
	CEN	CEN modified	CEN	CEN modified	CEN	CEN modified
0.01	0.0149	0.0173	0.0012	0.00095	8.04	5.48
0.02	0.0298	0.327	0.00155	0.00067	5.2	2.06
0.2	0.3072	0.3345	0.01793	0.00097	5.84	0.29
0.4	0.6272	0.6594	0.00857	0.0028	1.37	0.42
0.6	0.9357	0.9658	0.02494	0.01767	2.66	1.83

Figure 4: Comparison between Methods DIN 5314 and CEN LGR modification

No.	Leather type	Colour	DIN 5314					CEN/TS 14495 (IUC 18) LGR modification				
			Dilution	Absorbance	Absorbance difference	Cr(VI) mg/kg DM	Recovery rate %	Dilution	Absorbance	Absorbance difference	Cr(VI) mg/kg DM	LGR modification
1	Bovine Lining	Yellow	10/50	0.003	0.027	4.7	101	10/25/10/25	-0.001	0.020	n.d.	102
2	Gloving	Dark Violet	5/50	0.489	0.008	n.d. (< 6)	101	10/25/10/25	0.013	0.003	n.d.	77
3	Goat Suede	Yellowish	10/50	0.003	0.051	8.9	98	10/25/10/25	0.003	0.054	n.d.	95
4	Bovine Lining	Ochre	10/50	0.021	0.011	n.d.	94	10/25/10/25	0.003	0.003	n.d.	97
5	Watch Strap	Dark Grey	10/50	0.132	0.001	n.d.	103	10/25/10/25	0.008	0.001	n.d.	90
6	Watch Strap	Dark Brown	10/50	0.375	0.004	n.d.	88	10/25/10/25	0.012	0.004	n.d.	74
7	Bovine Suede	Brown	10/50	0.116	0.110	19.1	99	10/25/10/25	0.005	0.085	18.6	98
8	Goat Suede	Brown	10/50	0.141	0.082	13.8	98	10/25/10/25	0.027	0.061	12.9	
9	Upholstery	Blue	10/50	0.432	-0.001	n.d.	82	10/25/10/25	0.005	0.002	n.d.	91
10	Clothing	Red Brown	10/50	0.051	0.001	n.d.	82	10/25/10/25	0.005	0.002	n.d.	93
11	Watch Strap	Brown	10/50	0.168	0.002	n.d.	94	10/25/10/25	0.001	0.004	n.d.	97
12	Bovine Nubuck	Orange Red	10/50	0.026	0.004	n.d.	76	10/25/10/25	0.002	0.003	n.d.	85
13	Sheep Suede	Yellow	10/50	0.059	0.098	17.0	103	10/25/10/25	0.016	0.079	17.1	108
14	Goat Suede	Yellowish	10/50	0.001	0.542	92.9	103	10/25/10/25	-0.001	0.430	92.6	97
15	Sheep Grain	Black	5/50	0.382	0.063	21.7	73	10/25/10/25	0	0.072	15.6	95
16	Sheep Grain	Blue	10/50	0.099	0.297	50.7	97	10/25/10/25	-0.001	0.208	44.7	97
17	Sheep Grain	Black	5/50	0.394	0.004	n.d. (<6)	68	10/25/10/25	0.002	0.002	n.d.	95
18	Goat Suede	Black	2.5/50	0.533	0.010	n.d. (<12)	85	10/25/10/25	0.005	0.002	n.d.	76
19	Sheep Grain	Black	2.5/50	0.252	0.004	n.d. (<12)	82	10/25/10/25	0.007	0.007	n.d.	93
20	Goat Suede	Blue/Black	5/50	0.318	0.021	6.4	88	10/25/10/25	0.005	0	n.d.	93
21	Goat Suede	Yellow	10/50	0.017	0.144	24.3	109	10/25/10/25	0.001	0.113	23.9	110
22	Goat Nappa	Yellow	10/50	0.004	0.036	5.9	101	10/25/10/25	-0.002	0.024	n.d.	101
23	Bovine Split	Blue	10/50	0.094	0.350	52.0	98	10/25/10/25	0.001	0.278	53.1	105
24	Goat Suede	Orange Red	5/50	0.484	0.040	13.1	92	10/25/10/25	0.016	0.016	10.1	92
25	Goat Suede	Lilac	10/50	0.085	0.005	n.d.	109	10/25/10/25	0	0.004	n.d.	102
26	Bovine	Lilac	10/50	0.083	0.006	n.d.	112	10/25/10/25	0.001	0.005	n.d.	92
27	Sheep Grain	Ruby-coloured	2.5/50	0.586	0.022	13.1	89	10/25/10/25	0.048	0.004	n.d.	113
28	Bovine	Royal Blue	10/50	0.175	-0.003	n.d.	48	10/25/10/25	0.018	-0.001	n.d.	

With the CEN method calibration, it was not possible to achieve a quantification limit of 10 ppm. Using the statistical evaluation given in DIN 32645⁽⁶⁾, the quantification limit of the basic analytical procedure is 0.077µg Cr(VI)/ml (*confidence level 99%*). The same technician following the recommendations of items 2 & 3 achieved a detection limit of 0.01µg Cr(VI)/ml.

Similarly other statistical data is affected. The standard deviation of the CEN method is 0.00572µg/ml whilst the modified method 0.000723mg/ml. The residual standard deviation for the CEN method is 0.008832 absorbance units (abs), whereas for the modified method it is 0.000723 abs.

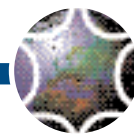
By changing the adding order of the reagents the calibration quality is noticeably improved. The time interval between

acidification and the addition of the DPC made no difference over the time span of 10 minutes (investigated time period).

Item 4

The precision was determined over the whole working range. For this purpose ten independent dilutions were prepared and measured for each calibration level. The measured values with the highest and lowest concentrations were already at the limits of the linear range of the spectrophotometer (*as shown in Figure 3*).

A comparison of the pH of the measured analytical solutions without the matrix indicated that the DIN solution presents pH 1.5 and the CEN solution pH 2.1 (*see Figure 1*). With double the amount of phosphoric acid the pH becomes 1.6. According to



Harzdorf⁽⁷⁾ the optimal pH of the reaction on which the procedure is based is between pH 0.7 and pH 1.3. In very acid solutions the coloured complex is no longer stable, and in weaker acid solutions its formation is slower.

When calibrations are carried out using different amounts of acid (0.5 or 1ml of phosphoric acid) no significant differences were found.

The linear measuring range of the spectrophotometer is limited both at the top and bottom (*Lambert-Beer law*). In quantification it should be noted that each element has a specific range. In the case of Cr(VI) in leather the concentration limiting the linear range at the bottom is of interest. The high molar absorbance coefficient of the coloured complex allows relatively low concentrations. The literature mentions a detection limit of 5 ppb⁽⁷⁾. But in routine analysis of Cr(VI) in leather only realistic values should be taken into account. If the absorbance is too small, the observed measuring errors become too high and the results will scatter widely⁽⁸⁾.

Recovery rate

Laboratory practice has shown that, according to the CEN procedure, the recovery rate gives rise in many cases to erroneous results. The reason for this is that for determining the recovery rate the spiked extract is bleached in a separate cartridge and its absorbance without DPC is not determined. Due to slight differences between cartridges the result of bleaching can differ. However, these discrepancies are not considered in the calculations of the recovery, hence false results arise. Therefore it is recommendable to determine the absorbance of the bleached spiked extract without the addition of DPC as well and to take this value into account in calculating the recovery rate.

DIFFERENCES IN OUTCOME BETWEEN PROCEDURES IN PRACTICE

Taking into account the modifications discussed, a comparison of the two procedures as given in *Figure 4* shows that there is considerable agreement between the two methods. The results were obtained using a cell length of 2 cm.

Only two of the samples (20 and 27) show a false positive according to DIN53314. This arises due to the interference of the dye colour (*note the lowering of the absorbance difference due to the bleaching stage of the CEN method*). In several cases the quantification limit of 3ppm cannot be achieved especially due to a changed dilution. Strongly coloured extracts must be diluted to

a greater extent in order to achieve the given value of the blank.

Conclusions

As a result of internal investigations and with the purpose to improve the efficiency, precision, and sensitivity of the chromate determination as method CEN/TS14495, LGR recommend the following changes to the standard:

- The minimum number of calibration concentrations should be 6.
- The blank solution should not be taken into consideration in plotting the calibration curve.
- The concentration range of the calibration should be within the linear measuring range.
- The chromate standards should be made using the buffer solution.
- The reagents for the development of the coloured complex should be added in the following order: first phosphoric acid and secondly diphenyl carbazide.
- In quoting the recovery rate the blank of the bleached spiked extract should be taken into consideration.

In view of these findings, the increase of the quantification limit of CEN/TS14495 compared with that of DIN 53314 is considered justified. The modifications proposed and maybe further improvement of the procedure could possibly lead to a lowering of the quantification limit. This remains to be established by round-robin tests within the appropriate standardising bodies. 🌐

References:

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