

J. Clin. Chem. Clin. Biochem.
Vol. 24, 1986, pp. 141–146

© 1986 Walter de Gruyter & Co.
Berlin · New York

Simultaneous Spectrophotometric Calibration of Wavelength and Absorbance in an Interlaboratory Survey Using Holmium Oxide (Ho_2O_3) in Perchloric Acid as Reference, Compared with *p*-Nitrophenol and Cobaltous Sulphate Solutions (1978–1984)

By A. P. Jansen

Clinical Chemical Laboratories, St. Radboud Hospital, University, Nijmegen, The Netherlands

E. J. van Kampen

Clinical Chemical Laboratories, Diaconessen Hospital, Groningen, The Netherlands

H. Steigstra

Clinical Chemical Laboratories, St. Radboud Hospital, University, Nijmegen, The Netherlands

P. H. W. van der Ploeg and A. Zwart

Clinical Chemical Laboratories, Diaconessen Hospital, Groningen, The Netherlands

(Received May 3/September 2, 1985)

Summary: The *wavelength accuracy* of ten different types of spectrophotometer was tested with holmium perchlorate solutions. It was found to be good, with mean deviations from the literature values of maximally 0.3 nm. Standard deviations over the entire spectral range were within 0.75 nm.

The *absorbance accuracy* for different types of instruments was generally within 5%, except in the 287 nm range where higher deviations were found. The sharpness of the holmium peaks, in combination with band width and sensitivity of the instruments, troubled the majority of the participants. 150 spectrophotometers were involved in the surveys.

Linearity of the spectrophotometers was tested with *p*-nitrophenol and cobaltous sulphate and found to be satisfactory.

*Gleichzeitige spektralphotometrische Kalibration von Wellenlänge und Absorbanz mit Holmiumoxid (Ho_2O_3)-Lösung in einem Ringversuch im Vergleich mit *p*-Nitrophenol und Cobaltsulfat (1978–1984)*

Zusammenfassung: Die *Richtigkeit der Wellenlänge* wurde bei verschiedenen Typen von Spektralphotometern mit Holmiumperchlorat Lösungen geprüft: Sie war gut, mit Abweichungen von maximal 0,3 nm von Mittelwerten aus der Literatur. Die Standardabweichungen über den gesamten Spektralbereich lagen innerhalb 0,75 nm.

Die *Richtigkeit der Absorbanz* für verschiedene Geräte-Typen war im allgemeinen innerhalb 5%, außer dem Bereich von 287 nm, wo höhere Abweichungen gefunden wurden. Die Schärfe der Holmium-Peaks in Zusammenhang mit der Bandbreite und der Empfindlichkeit der Geräte machte der Mehrheit der Teilnehmer Mühe.

Insgesamt wurden 150 Spektralphotometer untersucht. Die Linearität der Spektralphotometer wurde mit *p*-Nitrophenol und Cobaltsulfat geprüft und war zufriedenstellend.

Introduction

In spectrophotometry it is essential to calibrate absorbance (A) as well as wavelength (λ). Interlaboratory surveys for this evaluation in (clinical) chemistry laboratories have been performed only on a very limited scale. These surveys are usually restricted to the measurement of the absorbances of dichromate or cobaltous sulphate solutions (1–4). In the programmes of the German Society for Clinical Chemistry, absorbance checks at different wavelength are performed at regular intervals.

In the present survey we decided to use a holmium oxide solution in perchloric acid, because the absorption spectrum of holmium (Ho^{3+}) shows a great number of sharp peaks, providing an excellent means of calibrating the wavelength scale and the absorbance at the same time.

The sharpness of the peaks emphasized the need for narrow band pass filtering, and we attempted to demonstrate the errors that can arise from a lack of care in this area. We therefore purposely failed to meet one of *Rand's* criteria: broad absorption peaks (5), while adhering to his other criteria. Although few data are available from the literature about holmium absorption spectra (6, 7, 8) it is reasonable to expect that these data can be used comparatively in the present investigation. The spectrum (fig. 1) shows seven major peaks between 285 and 650 nm and we concentrated on these peaks.

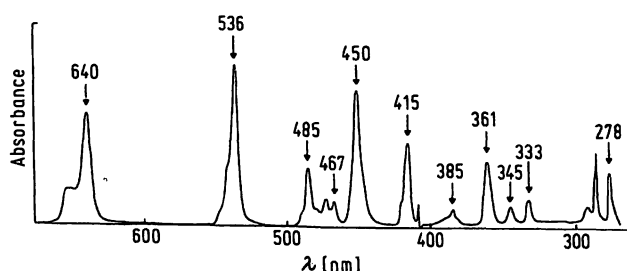


Fig. 1. The absorption-spectrum of Ho_2O_3 in perchloric acid solution.

Materials

In 1978 two *p*-nitrophenol solutions were distributed. Participants were requested to determine the wavelength at which maximum absorbance occurred and to measure the absorbance at 405 nm. The *p*-nitrophenol solutions were prepared by J. T. Baker Chemicals B. V., Deventer, The Netherlands at two concentrations with a weight ratio of 2.00. The maximum was established at 400.0 nm with a Cary spectrophotometer. The absorbances determined with the Cary were: solution A: 0.3178, solution B: 0.6281, ratio 1.976.

In 1983 two cobaltous sulphate solutions were prepared containing 10.0 g/l and 23.0 g/l, respectively. Participants were asked to establish the absorbances at 510 nm ($l = 10$ mm).

In the 1984 survey, solutions in perchloric acid, 0.1 mol/l with respect to holmium ions, (Ho^{3+}), were distributed in ampoules ad 5 ml. The solutions were prepared in the Clinical Chemical Laboratory of the Diaconessenhuis Groningen, by dissolving 18.90 g Ho_2O_3 (Ventron GmbH, Karlsruhe, F.R.G.; art. no. 89160, purity 99.999%) in 1 mol/l perchloric acid (≈ 100 g/l), and adjusting to 1 l. The light path is 10 mm. Thus the absorbances measured represent the fraction of 0.01 of molar lineic absorbance ($\text{m}^2 \cdot \text{mol}^{-1}$) at the given wavelengths ($\text{Ho}_2\text{O}_3 \rightarrow 2\text{Ho}^{3+}$).

The participants were asked to measure the holmium solution against aqua dest as a blank. The obligatory light path was 10.0 mm, and the measurements had to be performed under normal (routine) conditions. In the lower wavelength range quartz cuvettes must, of course, be used.

Results

In the measurement of the *p*-nitrophenol solutions the mean value for the maximal absorbance wavelength was 400.4 nm, with a standard deviation of 1.75 nm.

The absorbance measurements for the *p*-nitrophenol solutions and the ratio of the absorbances for the two different concentrations are given in table 1.

After elimination of outliers (> 3 s.d.) the mean absorbances of the cobaltous sulphate solutions were 0.1225 ± 0.0038 ($n = 132$) and 0.2819 ± 0.0068 ($n = 133$), giving coefficients of variation of 3.10% and 2.41%. The ratio of absorbances is 2.301.

For the holmium solutions the 7 major peaks (see fig. 1) are given in table 2 with the corresponding absorbances.

Tab. 1. Absorbance measurements in two *p*-nitrophenol solutions (1978 survey) at 405 nm.

Instrument	n	Solution A			Solution B		
		Absorbance	S.D.	C.V. (%)	Absorbance	S.D.	C.V. (%)
<i>Reference</i>							
Cary	1	0.6281			0.3178		
<i>Spectrophotometers</i>							
Perkin Elmer	18	0.6149	0.0241	3.9	0.3114	0.0212	6.8
Unicam	16	0.6163	0.0291	4.7	0.3129	0.0203	6.5
Beckman	60	0.6259	0.0189	3.0	0.3165	0.0125	3.9
Zeiss	32	0.6210	0.0190	3.1	0.3143	0.0172	5.5
Hitachi	10	0.6135	0.0362	5.9	0.3122	0.0119	3.8
Others	11	0.6227	0.0169	2.7	0.3135	0.0107	3.4
<i>Filter colorimeters</i>							
Vitatron	15	0.6014	0.0235	3.9	0.3064	0.0127	4.1
<i>Centrifugal analysers</i>							
Gemsaec, Centrifichem	9	0.6316	0.015	2.4	0.3190	0.0828	2.6
Total	171	0.6186	0.0218	3.5	0.3126	0.0146	4.7

Tab. 2. The seven major absorbance peaks for Ho^{3+} solutions with the corresponding absorbances. The absorbances correspond to the fraction of 0.01 of molar lineic absorbance (m^2/mol).

λ	S.D.	n	A	S.D.	C.V. (%)
287.4	0.78	126	0.215	0.067	31.2
361.1	0.65	151	0.197	0.020	10.2
416.3	0.70	153	0.237	0.017	7.2
451.3	0.78	154	0.387	0.012	3.1
485.2	0.73	146	0.164	0.018	11.0
536.7	0.73	153	0.454	0.025	5.5
640.7	0.77	144	0.312	0.012	3.8

154 spectrophotometers were used in this survey. In table 3 the different manufacturers are given. Subdivision into types yielded as many types as participants and is therefore not given.

A computer program was used for the evaluation of the data found by the participants in the seven major peaks. Values exceeding three standard deviations were excluded from the calculations; thereafter the mean values and standard deviations, and coefficients of variation were recalculated.

Discussion

Wavelength accuracy

The *p*-nitrophenol survey was only meant to check the wavelength calibration at the 400 nm region. The mean value obtained for the maximum absorbance

of the *p*-nitrophenol was in good agreement with the reference value of 400.0 nm. The conclusion might be that in this region the interlaboratory differences were rather small.

The holmium survey does confirm this conclusion. Not only is the wavelength calibration correct for the near visible region of the spectrum, but also for the whole visible spectrum. Moreover the standard deviation decreased to 0.75 nm. Thus 95% of the instruments measure within 1.5 nm of the maximum, which seems to be acceptable.

In figure 2 a histogram given for the 451.4 nm peak demonstrates that the spectrophotometers of different manufacturers are equally good. Table 3 compiles the results for all instruments at the different wavelengths. It is evident from this table that the wavelength accuracy for the different instruments is similar. Applying *Student's* t-distribution test, it can be shown that e. g. the mean wavelengths at 287 nm for the Beckman and Pye Unicam instruments are similar at the 5% significance level. And as instrumental differences in wavelength calibration over the entire spectrum do not exceed the mentioned difference at 287 nm, the given conclusion seems to be correct. It is obvious that in the scanning of the Ho^{3+} -spectrum the maxima can be found correctly without measuring the absorbances correctly. The maximal absorbance, although too low with respect to absolute values (due to the too large band-width of the instrument), still indicates accurately the wavelengths of peak absorbance.

Tab. 3. Wavelength accuracy for different instruments established with a holmium perchlorate solution.

Letter indication	Instrument	N	287 nm	361 nm	416 nm	451 nm	485 nm	536 nm	460 nm
A	Perkin Elmer	25	287.1 ± 0.90	360.9 ± 0.68	416.0 ± 0.70	451.0 ± 0.73	485.0 ± 0.75	536.4 ± 0.75	640.5 ± 0.99
B	Hitachi	9	287.3 ± 0.49	361.0 ± 0.50	416.4 ± 0.49	451.2 ± 0.49	485.2 ± 0.52	536.8 ± 0.44	640.7 ± 0.36
C	Beckman	49	287.8 ± 0.62	361.6 ± 0.64	416.6 ± 0.79	451.7 ± 0.75	485.6 ± 0.57	537.0 ± 0.56	641.0 ± 0.67
D	Shimadzu	7	287.1 ± 0.40	361.1 ± 0.18	416.1 ± 0.51	451.3 ± 0.40	485.4 ± 0.81	536.6 ± 0.70	640.2 ± 0.43
E	Pye Unicam	16	286.7 ± 0.63	360.8 ± 0.62	415.9 ± 0.58	451.0 ± 0.65	484.8 ± 0.69	536.2 ± 0.78	640.3 ± 0.81
F	Zeiss	21	287.7 ± 0.74	361.1 ± 0.78	416.6 ± 0.50	451.5 ± 0.83	485.5 ± 0.71	537.1 ± 0.81	641.5 ± 1.04
G	Optica	1	287.0	361.5	415.8	451.5	485.5	536.2	640.5
H	Bausch & Lomb	5	286.9 ± 0.73	360.7 ± 0.73	415.8 ± 0.78	450.8 ± 0.80	484.3 ± 0.55	536.6 ± 1.07	640.5 ± 0.20
I	Uvicon Kontron	9	287.1 ± 0.21	361.0 ± 0.29	416.2 ± 0.26	451.2 ± 0.39	485.0 ± 0.41	536.5 ± 0.27	640.7 ± 0.57
J	Hewlett Packard	1	287.0	361.0	416.0	450.0	484.0	536.0	642.0
K	Varian	4	287.2 ± 0.24	361.2 ± 0.24	416.2 ± 0.48	451.4 ± 0.79	485.0 ± 0.40	536.7 ± 0.28	640.6 ± 0.76
L	Gilford Stasar	4	287.7 ± 0.98	361.5 ± 0.50	417.0 ± 0.00	451.1 ± 1.51	484.8 ± 0.96	537.5 ± 0.71	640.8 ± 0.76
?	Other	3	287.6 ± 1.32	360.1 ± 0.14	415.9 ± 1.53	450.7 ± 0.58	484.5 ± 0.56	535.7 ± 0.75	640.4 ± 0.42
	Total survey	154	287.4 ± 0.78	361.1 ± 0.65	416.3 ± 0.70	451.3 ± 0.78	485.2 ± 0.73	536.7 ± 0.73	640.7 ± 0.77
	NBS study*		287.18	361.32	416.28	451.31	485.29	536.64	640.51

* see Addendum to the proof

Tab. 4. Mean absorbances of holmium perchlorate solution for different instruments at various wavelengths.

Letter indication	Instrument	N	A _{287 nm}	A _{361 nm}	A _{416 nm}	A _{451 nm}	A _{485 nm}	A _{536 nm}	A _{460 nm}
A	Perkin Elmer	25	0.216 ± 0.061	0.197 ± 0.023	0.236 ± 0.017	0.387 ± 0.012	0.161 ± 0.018	0.455 ± 0.019	0.313 ± 0.008
B	Hitachi	9	0.168 ± 0.052	0.182 ± 0.017	0.222 ± 0.017	0.376 ± 0.014	0.153 ± 0.015	0.431 ± 0.024	0.306 ± 0.012
C	Beckman	49	0.225 ± 0.062	0.198 ± 0.020	0.237 ± 0.020	0.388 ± 0.014	0.168 ± 0.019	0.456 ± 0.029	0.310 ± 0.013
D	Shimadzu	7	0.251 ± 0.096	0.204 ± 0.030	0.234 ± 0.014	0.387 ± 0.005	0.173 ± 0.023	0.476 ± 0.031	0.316 ± 0.016
E	Pye Unicam	16	0.224 ± 0.063	0.203 ± 0.018	0.238 ± 0.013	0.386 ± 0.012	0.168 ± 0.012	0.453 ± 0.020	0.314 ± 0.009
F	Zeiss	21	0.178 ± 0.076	0.189 ± 0.030	0.240 ± 0.013	0.393 ± 0.008	0.162 ± 0.021	0.448 ± 0.026	0.308 ± 0.024
G	Optica	1	0.292	0.216	0.254	0.392	0.183	0.482	0.322
H	Bausch & Lomb	5	0.200 ± 0.092	0.184 ± 0.027	0.221 ± 0.024	0.376 ± 0.018	0.153 ± 0.021	0.427 ± 0.048	0.304 ± 0.015
I	Uvicon Kontron	9	0.189 ± 0.019	0.195 ± 0.009	0.237 ± 0.007	0.394 ± 0.010	0.163 ± 0.005	0.453 ± 0.008	0.316 ± 0.010
J	Hewlett Packard	1	0.208	0.198	0.186	0.372	0.143	0.394	—
K	Varian	4	0.268 ± 0.104	0.201 ± 0.037	0.232 ± 0.033	0.392 ± 0.009	0.168 ± 0.027	0.447 ± 0.047	0.307 ± 0.018
L	Gilford Stasar	4	—	—	—	—	0.179	0.465	—
?	Other	3	0.240 ± 0.078	0.218	0.232 ± 0.020	0.387 ± 0.004	0.156 ± 0.019	0.449 ± 0.019	0.308 ± 0.003
	Reference (6)		0.337	0.220	0.255	0.394	0.185	0.442	0.317
	Reference (7)		0.331	0.218	0.252	0.400	0.458	0.458	0.305

λ (nm)	Spectrophotometer type
>453.4	A A C D E F
453.2	C L
453.0	C F
452.8	C
452.6	C C C C F
452.4	C
452.2	C C C C C C E F K
452.0	A A B C C C C C E F F F F F F I K
451.8	A A A C C C C C D E F F H
451.6	A A A B B B C C C D D E G
451.4	B C C F H
451.2	A A A B B C C C D D E F H I I I I I
451.0	A A C C C C C C C E E E E F F F F F I I K L ?
450.8	A A A E E
450.6	A A B C C D I K
450.4	A B E
450.2	
450.0	A A C C C E E F F F H H J L L ?
449.8	
449.6	A A
<449.4	

Fig. 2. Distribution of results for the $\lambda = 451.4$ nm peak of the holmium perchlorate solution. The letters indicate the different types of spectrophotometers (see tab. 3).

Absorbance measurements

The results obtained with the *p*-nitrophenol solutions (tab. 1) show that linearity is quite satisfactory in the 400 nm range. The difference between the mean value of the ratios of all results and the reference value was 0.1%.

For the individual spectrophotometers, Hitachi deviated most from the reference ratio, but even this deviation was only 0.55%. The Vitatron colorimeters showed a difference from the reference ratio of 0.65%. Not only the linearity is satisfactory in this 400 nm region, but also the absorbances themselves. The greatest difference in absorbance from the mean value, 2.3%, is scored again by Hitachi. The absorbances found with the Vitatron colorimeters, although showing slightly decreased values, agree pretty well with the reference values recorded with the Cary spectrophotometer: differences being within 4.3%. The results with the cobaltous sulphate solution in the 510 nm region are quite similar to those obtained a few years earlier with *p*-nitrophenol: the linearity is excellent with a difference between the mean ratio of absorbances and the expected value of less than 0.05%. The values obtained for the absorbances show coefficients of variation of 2.4–3.1%, representing a slight improvement with respect to the values found a few years earlier for the *p*-nitrophenol solutions.

The absorbances measured with the holmium solution in the visible part of the spectrum exhibit poorer results than those obtained for the *p*-nitrophenol and cobaltous sulphate solutions, with coefficients of variation varying from 3.8–10.8%. However it should

be kept in mind that the sharpness of the holmium peaks makes higher demands on instruments with regard to the combination of band width and sensitivity (tab. 4). These requirements are even stricter in the ultraviolet region of the spectrum, where the results are sometimes bewildering, e. g. at 287 nm, especially when comparing results with the reference values from the literature. Obviously sensitivity is too small to measure with minimal band width, using "real" monochromatic light. Figure 3 gives the individual results for absorbances at this wavelength. There is a slight indication that two populations exist in the distribution of results: one at $A = 0.320$, the other at 0.200.

$A_{287\text{ nm}}$	Spectrophotometer type
0.400	
0.380	
0.360	AC
0.340	CDDHK
0.320	CCDEEK?
0.300	ACEFFG
0.280	AAFK
0.260	ABCCE
0.240	AAABCCCCCCCCCCCCCF
0.220	ACCCCEEEEF?
0.200	AACCCEEEFIJ
0.180	AAAAABDEEFFIII
0.160	AACCCDDFFFFHHI?
0.140	ABBBCH
0.120	ACFFK
0.100	BCFF
0.080	EF
0.060	C
0.040	F
0.020	
0.000	

Fig. 3. Distribution of absorbances at 287 nm. The letters refer to different types of spectrophotometers (tab. 4).

The good performers (20% of total participants) concentrate around the former value, the weak performers around the latter.

Comparing the different types of instruments it is remarkable that *all* types are represented in the best-performing group, except Hitachi and Uvicon. Even more striking, is the fact that group of Uvicon-users shows the lowest standard deviations for the mean absorbances over the entire spectrum which is a good example of "precision". The mean absorbance values for the different wavelengths for Uvicon Kontron are in good agreement with the total mean values, except for the wavelength of 287.4 nm. Here the absorbance result is lower than the mean values of other types of instruments like Perkin Elmer, Beckman, Shimadzu and Pye Unicam, although the differences are not significant at the $P = 0.05$ level, owing to the relatively high standard deviations. Hitachi scores low absorbances over the entire wavelength range. Only 4 users of Gilford Stasar apparatus participated in the trial and it is therefore difficult to draw conclusions; nevertheless it is noteworthy that for all wavelengths the results of these participants had to be excluded from the calculations, because they exceeded 3 s. d.

Further the relatively high number of outliers for Zeiss users (from 25–50%) is striking. However, since the remainder of the Zeiss users score results comparable to the other values, this fact tells more about the user than about the instrument. It also illustrates that much care is always required in this type of instrument assessment.

References

- Edisbury, J. R. (1965) Photoelectr. Spectrometry Group Bull. 16, 441.
- Beeler, M. F. (1974) Am. J. Clin. Pathol. 61, 789.
- Vanderlinde, R. E., Richards, A. H. & Kowalski, P. (1975) Clin. Chim. Acta 61, 39–46.
- Stevens, J. F. (1980) Ann. Clin. Biochem. 17, 87–90.
- Rand, R. N. (1969) Clin. Chem. 15, 839–863.
- Klixbüll Jørgensen, Chr. (1957) Acta Chem. Scand. 11, 981–989.
- Banks, C. V. & Klingman, D. W. (1956) Anal. Chim. Acta 15, 356.
- Stewart, D. C. & Kato, D. (1958) Anal. Chem. 30, 164.

Prof. Dr. A. P. Jansen
Foundation for Quality Assessment
in Clinical Chemistry,
Hospital Laboratories (S. K. Z. L.)
St. Radboud Ziekenhuis
NL-6500 HB Nijmegen

Addendum to the proof

During editorial handling of our paper we discovered a reference study from the National Bureau of Standards, using holmium oxide in the same way as we did.

In the table we compare results from the N.B.S., published in the Journal of Research of the National Bureau of Standards, Vol. 90, Number 2, April 1985, with results of this survey. The agreement is excellent.

General conclusions

- Wavelength accuracy for different types of spectrophotometers is quite satisfactory with standard deviations of about 0.7 nm over the entire wavelength range.
- Linearity of spectrophotometers in the 400 and 500 nm regions is satisfactory. Such measurements were not performed in other wavelength ranges.
- Absorbance accuracy, especially at lower wavelengths (287 nm), depends strongly on handling of instruments. Absolute absorbance measurements in this wavelength range require a combination of high monochromaticity and high instrument sensitivity. Many users are obviously not aware of this fact, and measure absorbances that are too low.
- With the exception of the 287 nm region, absorbances of different types of instruments were within 10% of reference values and at the higher wavelengths even within 5%.
- The different type of instruments are equally satisfactory, although users of Gilford Stasar found in general absorbances that were too low. The 9 participants with a Uvicon Kontron excelled in the high precision of their absorbance measurements.

Pro memory

Laboratories interested in the Ho^{3+} solutions (5 ml) for checking their own instruments have a limited opportunity to order 1 or 2 ampoules at cost price.

Absorbance peak	λ (nm) NBS at 1 nm bandwidth	λ (nm) this survey
4	287.18	287.4
7	361.32	361.1
9	416.28	416.3
10	451.31	451.3
12	485.29	485.2
13	536.64	536.7
14	640.51	640.7