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Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics —

Part 2:

Calibration strategy for non-linear second-order calibration functions

Qualité de l'eau — Étalonnage et évaluation des méthodes d'analyse et estimation des caractères de performance —

Partie 2: Stratégie d'étalonnage pour fonctions d'étalonnage non linéaires du second degré

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this part of ISO 8466 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 8466-2 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical, biochemical methods*.

This second edition cancels and replaces the first edition (ISO 8466-2:1993), which has been technically revised.

ISO 8466 consists of the following parts, under the general title *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics*:

- *Part 1: Statistical evaluation of the linear calibration function*
- *Part 2: Calibration strategy for non-linear second-order calibration functions*

Annex A forms an integral part of this part of ISO 8466.

INTERNATIONAL STANDARD **ISO 8466-2:2001(E)**

Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics —

Part 2:
Calibration strategy for non-linear second-order calibration functions

1 Scope

It is not always possible to accurately describe the relationship between a set of calibration points with a rectilinear curve, even by decreasing the working range. Instead of the linear regression analysis, a least-squares fit to a second-order polynomial is applied (see test for linearity in 4.1.3 of ISO 8466-1:1990[1]). Using this fit, it is possible to calculate not only the calibration function but also the confidence interval associated with it.

This part of ISO 8466 is intended primarily for use in method development and may not necessarily be applicable to all routine analyses.

2 Symbols

a, b, c	Coefficients of the calibration function
E	Instrument sensitivity in the centre of the working range
e	Instrument sensitivity, defined as the first derivative of the calibration function
$F(f_1, f_2, P)$	Tabled value of the F-distribution with f_1 and f_2 degrees of freedom and a confidence level of P (%)
F_{calc}	Test value calculated for the F-test
f	Number of degrees of freedom for the residual standard deviation ($f = N - 3$)
$I(\hat{x})$	Confidence interval for the concentration $\hat{x}$
i	Subscript of the concentration levels, where i = 1, 2, ..., N
j	Subscript of the replicates of level i, where j = 1, 2, ..., n_i
N	Number of concentration levels (for this part of ISO 8466, N = 10)
$\hat{N}$	Number of replicates for the same analytical sample
n_i	Number of replicates per level x_i
$s_{x\text{o}}$	Standard deviation of the procedure

s_y	Residual standard deviation
s_i^2	Variance of the response variable values for the analyses of standard samples, having the concentration x_i
$t(f_1, P)$	Tabled value of the t-distribution with $f_1 = N - 3$ degrees of freedom and a confidence interval of P (%) (t-factor of Student's distribution)
$V_{x\mathrm{o}}$	Coefficient of variation of the procedure
x_1	Concentration of the standard sample at the lower level of the working range (1st standard sample)
x_{10}	Concentration of the standard sample at the upper level of the working range (10th standard sample)
x_i	Concentration of the ith standard sample
$\overline{x}$	Mean of the standard concentrations x_i, resulting from the calibration experiment
x^*	Concentration at which the calibration function has a minimum or a maximum value
$\hat{x}$	Concentration of the analytical sample, calculated from the response variable value $\hat{y}$
$y_{i,j}$	jth response variable value for the concentration x_i
$\overline{y}$	Mean of the response variable values y_i, resulting from the calibration experiment
$\overline{y}_i$	Mean of the response variable values $y_{i,j}$ of standard samples, having the concentration x_i
$\hat{y}$	Response variable value of an analytical sample
$\hat{y}_i$	Response variable value of the standard concentration x_i calculated from the calibration function

3 Performance

3.1 Choice of working range

The calibration experiment requires that the preliminary working range be established according to the following.

a) Define the objective of the calibration with respect to the practical application.

The working range may cover the application range required for the analysis of water, wastewater and sludge. The most frequently expected sample concentration should lie near the centre of the working range.

b) Make sure the values obtained near the lower limit of the working range are distinguishable from the blanks of the procedure.

The lower limit of the working range should therefore be equal to or greater than the limit of quantitation of the procedure. Dilution or concentration steps should be feasible and free from the risk of bias.

3.2 Test for the homogeneity of the variances

The variance of the values determined from selected standard solutions shall be homogeneous and independent of the concentration.

After establishing the preliminary working range, determine the response variable values of at least $N = 5$ (recommended $N = 10$) standard solutions. Select the standard solution concentrations, x_i, so as to distribute them equidistantly over the working range. In order to examine the homogeneity of the variances, s_i^2, make replicate measurements of each of the lowest and highest concentrations of the standard solutions of the working range. n_i values ($y_{i,j}$) result from these series of measurements.

Both data sets of the concentrations x_1 and x_{10} are used to calculate the variances s_1^2 and s_{10}^2 as given in equation (1):

$$s_i^2 = \frac{\sum_{j=1}^{n_i} \left(y_{i,j} - \overline{y}_i\right)^2}{n_i - 1} \tag{1}$$

where

f_i $= n_i - 1$

$\overline{y}_i$ is the mean as given in Equation (2)

$$\overline{y}_i = \frac{\sum_{j=1}^{n_i} y_{i,j}}{n_i} \tag{2}$$

for $i = 1$ or $i = 10$.

The variances are submitted to a simple test of variance (one-sided F-test), in order to examine for significant differences at the lower and upper limits of the working range.

Determine the test value F_{calc} as follows:

$$F_{\text{calc}} = \frac{s_{10}^2}{s_1^2} \quad \text{for} \quad s_{10}^2 > s_1^2 \tag{3}$$

$$F_{\text{calc}} = \frac{s_1^2}{s_{10}^2} \quad \text{for} \quad s_1^2 > s_{10}^2 \tag{4}$$

Compare the value of F_{calc} with the tabled values of the F-distribution given in Table A.1 of annex A.

Make the following decision:

a) if $F_{\text{calc}} \leqslant F(f_1, f_2, 99\ \%)$, the difference between the variances is not significant;

b) if $F_{\text{calc}} > F(f_1, f_2, 99\ \%)$, the difference between the variances is significant.

In the case of b), decrease the preliminary working range until the difference between the variances is found to be random only.

3.3 Measurement

After the establishment of the final working range, prepare $N = 10$ standard solutions whose concentrations x_i are equidistantly distributed over the working range. Measure the respective values of the response variable, y_i.

4 Estimation of the polynomial coefficients

Using the values of the standard concentrations as independent variables and the measured values as dependent or response variables, calculate the calibration coefficients of the polynomial using Equation (5) as follows:

$$y = a + bx + cx^2 \tag{5}$$

The following intermediate values are required for the calculation of the coefficients a, b and c:

$$Q_{xx} = \sum x_i^2 - \frac{\left(\sum x_i\right)^2}{N} \tag{6}$$

$$Q_{xy} = \sum (x_i y_i) - \left(\sum x_i \times \frac{\sum y_i}{N} \right) \tag{7}$$

$$Q_{x^3} = \sum x_i^3 - \left(\sum x_i \times \frac{\sum x_i^2}{N} \right) \tag{8}$$

$$Q_{x^4} = \sum x_i^4 - \frac{\left(\sum x_i^2\right)^2}{N} \tag{9}$$

$$Q_{x^2 y} = \sum \left(x_i^2 \times y_i \right) - \left(\sum y_i \times \frac{\sum x_i^2}{N} \right) \tag{10}$$

The centre of the working range is given by:

$$\bar{x} = \frac{\sum x_i}{N} \tag{11}$$

The average of the values of the response variable, y_i, is given by:

$$\bar{y} = \frac{\sum y_i}{N} \tag{12}$$

Estimate the coefficients of the equation of the calibration function as follows:

$$c = \frac{\left(Q_{xy} \times Q_{x^3}\right) - \left(Q_{x^2 y} \times Q_{xx}\right)}{\left(Q_{x^3}\right)^2 - \left(Q_{xx} \times Q_{x^4}\right)} \tag{13}$$

$$b = \frac{Q_{xy} - cQ_{x^3}}{Q_{xx}} \tag{14}$$

$$a = \frac{\left(\sum y_i - b\sum x_i - c\sum x_i^2\right)}{N} \quad (15)$$

In order to test the adequacy of the second-order function, the residues $\left(y_i - \hat{y}_i\right)$ should be plotted against the respective concentration values.

Because of unavoidable procedural random errors, the calculated coefficients of the calibration functions can be regarded as estimates only. Their precision is quantified by the residual standard deviation s_y. This is a standard deviation which quantitatively describes the scatter of the response variable values y around the second-order function.

5 Performance characteristics

5.1 Residual standard deviation

The residual standard deviation, s_y, is calculated from Equation (16).

$$s_y = \sqrt{\frac{\sum \left(y_i - \hat{y}_i\right)^2}{N-3}} \quad (16)$$

where

$$\hat{y}_i = a + bx_i + cx_i^2 \quad (17)$$

or

$$s_y = \sqrt{\frac{\sum y_i^2 - a\sum y_i - b\sum x_i y_i - c\sum x_i^2 y_i}{N-3}} \quad (18)$$

with the number of degrees of freedom equal to:

$$f = N - 3 \quad (19)$$

5.2 Instrument sensitivity of the analytical procedure

The instrument sensitivity (referred to as simply "sensitivity" hereafter) is derived from the change of the response variable value resulting from a change of the concentration value. In the case of a rectilinear calibration function, the sensitivity is constant over the entire working range and is represented by the regression coefficient b[2]. In the event of a non-linear calibration function, the sensitivity e is given by the first derivative of the calibration function as follows:

$$e = b + 2cx \quad (20)$$

The sensitivity in the centre $\overline{x}$ of the working range is given as a characteristic of the procedure:

$$E = b + 2c\overline{x} \quad (21)$$

where E is the slope (tangent) of the calibration function at the centre $\overline{x}$ of the working range.

5.3 Standard deviation of the procedure

The standard deviation of the procedure s_{x0} is derived from the residual standard deviation s_y and the sensitivity E. This is an unambiguous figure of merit for the assessment of the analytical procedure.

The standard deviation of the procedure is given by Equation (22).

$$s_{x0} = \frac{s_y}{E} \tag{22}$$

The standard deviation of the procedure s_{x0} (with $f = N - 3$ degrees of freedom) can be used for the comparison of analytical procedures, provided N and the working range are constant and the calibration standards are equidistantly distributed within the working range.

5.4 Relative standard deviation of the procedure

The relative standard deviation of the procedure, V_{x0}, allows a comparison of the performance of analytical procedures and is calculated, as a percentage, using Equation (23):

$$V_{x0} = \frac{s_{x0} \times 100}{\bar{x}} \tag{23}$$

6 Analysis of a sample

6.1 General considerations

The following conditions are necessary to obtain an accurate and precise result.

The calibration function shall not have a maximum or minimum within the working range. Maxima or minima can be discovered on the basis of the sensitivity e (concentration-dependent sensitivity). If the sensitivity (the slope of the calibration function) becomes zero at any point x^*, it can be concluded that the function is not unambiguously defined and therefore the calculated second-order function shall not be used.

6.2 Test for minima or maxima

Using Equation (20)

$$e = b + 2cx$$

and

$$x^* = -\frac{b}{2c} \quad \text{for} \quad e^* = 0 \tag{24}$$

Test:

- If $x_1 < x^* < x_{10}$, the function is not single-valued due to a maximum or a minimum within the working range and cannot be used for further evaluation of the analytical results.
- If $x^* < x_1$ or $x^* > x_{10}$, the calibration function is single-valued and can be used for further evaluation of the analytical results.

6.3 Calculation of the analytical result

In order to obtain the concentration value $\hat{x}$ from the measured value (response variable value) $\hat{y}$, it is necessary to determine the inverse function of Equation (5) as follows.

- For positively-curved calibration functions, apply Equation (25):

$$\hat{x} = \frac{-b}{2c} + \sqrt{\left(\frac{b}{2c}\right)^2 - \frac{a-\hat{y}}{c}} \qquad (25)$$

— For negatively-curved calibration functions, apply Equation (26):

$$\hat{x} = \frac{-b}{2c} - \sqrt{\left(\frac{b}{2c}\right)^2 - \frac{a-\hat{y}}{c}} \qquad (26)$$

6.4 Confidence interval of the analytical result (see Figure 1)

It has to be taken into account that the analytical error does not only consist of the error originating with the determination of the response variable value, but also of the error s_y, originating in the calibration function[3].

The law of error propagation shall therefore be applied to the estimation of the confidence interval of the analytical result. The width of the confidence interval depends on the following parameters:

a) residual standard deviation s_y;

b) number N of standard solutions used for the calibration;

c) number $\hat{N}$ of replicate measurements made on the unknown sample;

d) sensitivity e of the analytical procedure at the concentration $\hat{x}$;

e) distance of the analytical result from the average of the standard solution concentrations, $\hat{x} - \bar{x}$.

The approximation of the confidence interval $I(\hat{x})$ is given by:

$$I(\hat{x}) = \frac{s_y \cdot t_{f1,P}}{\left(b + 2c\hat{x}\right)} \cdot \sqrt{\frac{1}{N} + \frac{1}{\hat{N}} + \frac{\left(\hat{x}-\bar{x}\right)^2 Q_{x^4} + \left(\hat{x}^2 - \frac{\sum x_i^2}{N}\right)^2 Q_{xx} - 2\left(\hat{x}-\bar{x}\right)\left(\hat{x}^2 - \frac{\sum x_i^2}{N}\right) Q_{x^3}}{Q_{x^4} Q_{xx} - \left(Q_{x^3}\right)^2}} \qquad (27)$$

The analytical result is given by:

$$\hat{x}_{1,2} = \hat{x} \pm I(\hat{x}) \qquad (28)$$

In order to guard against rounding errors, it is recommended to use as many decimal places as possible in the calculation.

7 Example

7.1 Calibration, performance characteristics and evaluation

Analytical procedure: hypothetical example given in Figure 2.

Working range: 12 mg/l to 66 mg/l.

The homogeneity of the variances was proven by preliminary tests.

Calibration data are given in Table 1.

Checking, in accordance with 6.2, for the maxima or minima within the working range gives:

$$x^* = -\frac{b}{2c} \tag{29}$$

$$x^* = -\frac{0{,}007\,67}{2\left(-0{,}000\,025\right)} = 153{,}2 \text{ mg/l} \tag{30}$$

Decision: The condition $x^* > x_{10}$ is met; the calibration function is therefore valid.

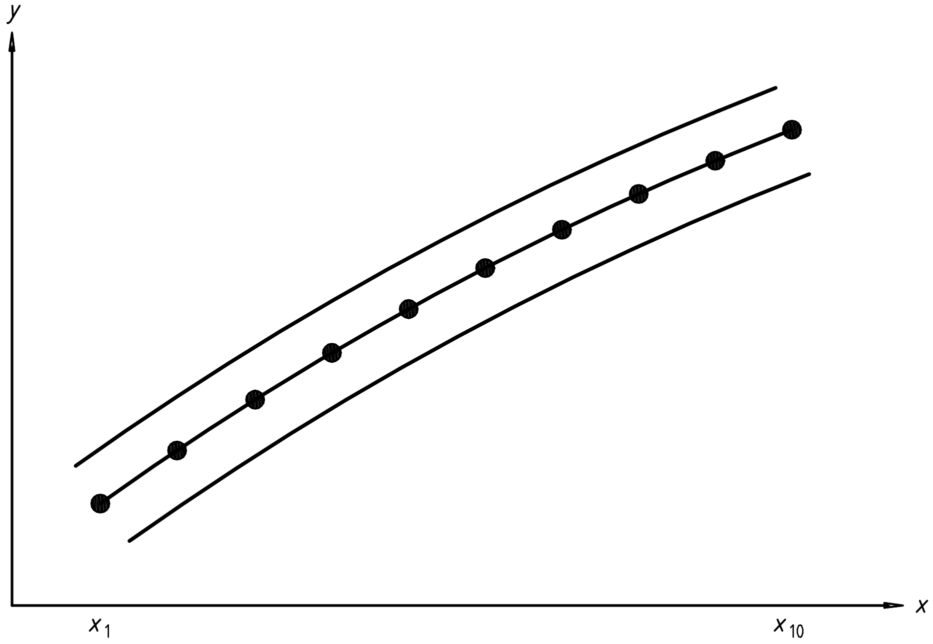

Figure 1 — Second-order calibration function for the confidence interval (x_1 to x_{10})

7.2 Single measurement analysis

The response variable value (absorbance) of a sample is:

$$\hat{y} = 0{,}084$$

Calculation of the analytical result using Equation (26) gives the concentration of the sample as:

$$\hat{x} = 12{,}17 \text{ mg/l}$$

The 95 %-confidence interval [$t(95\ \%; f = 7) = 2{,}36$] is:

$$I(\hat{x}) = \pm\ 0{,}63 \text{ mg/l}$$

The result is:

$$\hat{x}_{1,2} = \left(12{,}17 \pm 0{,}63\right) \text{ mg/l}$$

The true value of the unknown concentration is expected to be between 11,54 mg/l and 12,80 mg/l.

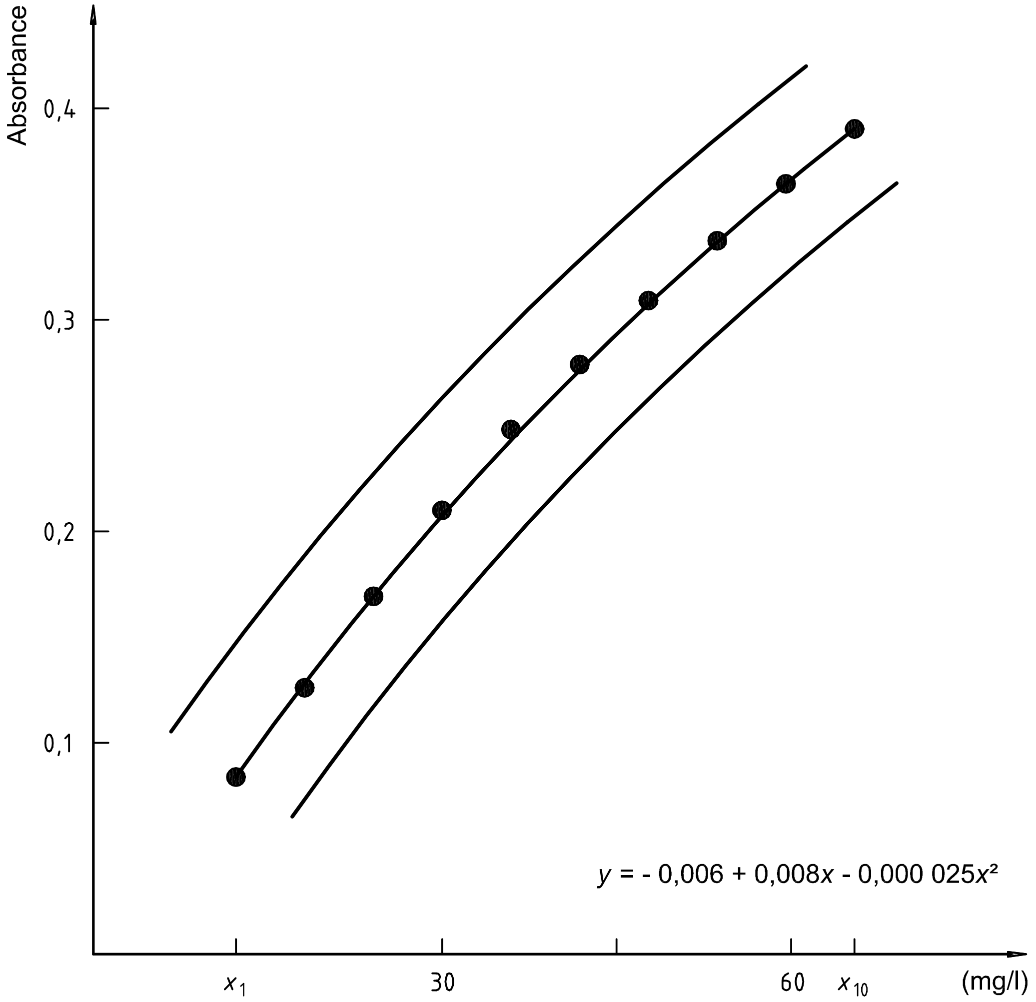

Figure 2 — Hypothetical example of a second-order calibration function

Table 1 — Calibration data

Concentration x_i mg/l	**Absorbance** y_i
12	0,083
18	0,123
24	0,164
30	0,203
36	0,240
42	0,273
48	0,303
54	0,334
60	0,364
66	0,393

a = – 0,005 62

b = 0,007 67 l/mg

c = – 0,000 025 l^2/mg^2

s_y = 0,001 48

$s_{x\text{o}}$ = 0,258 62 mg/l (at concentration $\overline{x}$)

$V_{x\text{o}}$ = 0,66 %

Annex A
(normative)

F-table (99 %)

Values of the F-distribution (99 %) are given in Table A.1.

Table A.1 — Values of the F-distribution (99 %)

		Degrees of freedom for larger variance																	
	f_2/f_1	1	2	3	4	5	6	7	8	9	10	12	15	20	24	30	40	60	120	∞
Degrees of freedom for smaller variance	1	4 052	4 999,5	5 403	5 625	5 764	5 859	5 928	5 982	6 022	6 056	6 106	6 157	6 209	6 235	6 261	6 287	6 313	6 339	6 366
	2	98,50	99,00	99,17	99,25	99,30	99,33	99,36	99,37	99,39	99,40	99,42	99,43	99,45	99,46	99,47	99,47	99,48	99,49	99,50
	3	34,12	30,82	29,46	28,71	28,24	27,91	27,67	27,49	27,35	27,23	27,05	26,87	26,69	26,60	26,50	26,41	26,32	26,22	26,13
	4	21,20	18,00	16,69	15,98	15,52	15,21	14,98	14,80	14,66	14,55	14,37	14,20	14,02	13,93	13,84	13,75	13,65	13,56	13,46
	5	16,26	13,27	12,06	11,39	10,97	10,67	10,46	10,29	10,16	10,05	9,89	9,72	9,55	9,47	9,38	9,29	9,20	9,11	9,02
	6	13,75	10,92	9,78	9,15	8,75	8,47	8,26	8,10	7,98	7,87	7,72	7,56	7,40	7,31	7,23	7,14	7,06	6,97	6,88
	7	12,25	9,55	8,45	7,85	7,46	7,19	6,99	6,84	6,72	6,62	6,47	6,31	6,16	6,07	5,99	5,91	5,82	5,74	5,65
	8	11,26	8,65	7,59	7,01	6,63	6,37	6,18	6,03	5,91	5,81	5,67	5,52	5,36	5,28	5,20	5,12	5,03	4,95	4,86
	9	10,56	8,02	6,99	6,42	6,06	5,80	5,61	5,47	5,35	5,26	5,11	4,96	4,81	4,73	4,65	4,57	4,48	4,40	4,31
	10	10,04	7,56	6,55	5,99	5,64	5,39	5,20	5,06	4,94	4,85	4,71	4,56	4,41	4,33	4,25	4,17	4,08	4,00	3,91
	11	9,65	7,21	6,22	5,67	5,32	5,07	4,89	4,74	4,63	4,54	4,40	4,25	4,10	4,02	3,94	3,86	3,78	3,69	3,60
	12	9,33	6,93	5,95	5,41	5,06	4,82	4,64	4,50	4,39	4,30	4,16	4,01	3,86	3,78	3,70	3,62	3,54	3,45	3,36
	13	9,07	6,70	5,74	5,21	4,86	4,62	4,44	4,30	4,19	4,10	3,96	3,82	3,66	3,59	3,51	3,43	3,34	3,25	3,17
	14	8,86	6,51	5,56	5,04	4,69	4,46	4,28	4,14	4,03	3,94	3,80	3,66	3,51	3,43	3,35	3,27	3,18	3,09	3,00
	15	8,68	6,36	5,42	4,89	4,56	4,32	4,14	4,00	3,89	3,80	3,67	3,52	3,37	3,29	3,21	3,13	3,05	2,96	2,87
	16	8,53	6,23	5,29	4,77	4,44	4,20	4,03	3,89	3,78	3,69	3,55	3,41	3,26	3,18	3,10	3,02	2,93	2,84	2,75
	17	8,40	6,11	5,18	4,67	4,34	4,10	3,93	3,79	3,68	3,59	3,46	3,31	3,16	3,08	3,00	2,92	2,83	2,75	2,65
	18	8,29	6,01	5,09	4,58	4,25	4,01	3,84	3,71	3,60	3,51	3,37	3,23	3,08	3,00	2,92	2,84	2,75	2,66	2,57
	19	8,18	5,93	5,01	4,50	4,17	3,94	3,77	3,63	3,52	3,43	3,30	3,15	3,00	2,92	2,84	2,76	2,67	2,58	2,49
	20	8,10	5,85	4,94	4,43	4,10	3,87	3,70	3,56	3,46	3,37	3,23	3,09	2,94	2,86	2,78	2,69	2,61	2,52	2,42
	21	8,02	5,78	4,87	4,37	4,04	3,81	3,64	3,51	3,40	3,31	3,17	3,03	2,88	2,80	2,72	2,64	2,55	2,46	2,36
	22	7,95	5,72	4,82	4,31	3,99	3,76	3,59	3,45	3,35	3,26	3,12	2,98	2,83	2,75	2,67	2,58	2,50	2,40	2,31
	23	7,88	5,66	4,76	4,26	3,94	3,71	3,54	3,41	3,30	3,21	3,07	2,93	2,78	2,70	2,62	2,54	2,45	2,35	2,26
	24	7,82	5,61	4,72	4,22	3,90	3,67	3,50	3,36	3,26	3,17	3,03	2,89	2,74	2,66	2,58	2,49	2,40	2,31	2,21
	25	7,77	5,57	4,68	4,18	3,85	3,63	3,46	3,32	3,22	3,13	2,99	2,85	2,70	2,62	2,54	2,45	2,36	2,27	2,17
	26	7,72	5,53	4,64	4,14	3,82	3,59	3,42	3,29	3,18	3,09	2,96	2,81	2,66	2,58	2,50	2,42	2,33	2,23	2,13
	27	7,68	5,49	4,60	4,11	3,78	3,56	3,39	3,26	3,15	3,06	2,93	2,78	2,63	2,55	2,47	2,38	2,29	2,20	2,10
	28	7,64	5,45	4,57	4,07	3,75	3,53	3,36	3,23	3,12	3,03	2,90	2,75	2,60	2,52	2,44	2,35	2,26	2,17	2,06
	29	7,60	5,42	4,54	4,04	3,73	3,50	3,33	3,20	3,09	3,00	2,87	2,73	2,57	2,49	2,41	2,33	2,23	2,14	2,03
	30	7,56	5,39	4,51	4,02	3,70	3,47	3,30	3,17	3,07	2,98	2,84	2,70	2,55	2,47	2,39	2,30	2,21	2,11	2,01
	40	7,31	5,18	4,31	3,83	3,51	3,29	3,12	2,99	2,89	2,80	2,66	2,52	2,37	2,29	2,20	2,11	2,02	1,92	1,80
	60	7,08	4,98	4,13	3,65	3,34	3,12	2,95	2,82	2,72	2,63	2,50	2,35	2,20	2,12	2,03	1,94	1,84	1,73	1,60
	120	6,85	4,79	3,95	3,48	3,17	2,96	2,79	2,66	2,56	2,47	2,34	2,19	2,03	1,95	1,86	1,76	1,66	1,53	1,38
	∞	6,63	4,61	3,78	3,32	3,02	2,80	2,64	2,51	2,41	2,32	2,18	2,04	1,88	1,79	1,70	1,59	1,47	1,32	1,00

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