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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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Contents

	Page
1 Scope	1
2 Symbols	1
3 Performance	2
3.1 Choice of working range	2
3.2 Test for the homogeneity of the variances	2
3.3 Measurement	3
4 Estimation of the polynomial coefficients	3
5 Performance characteristics	4
5.1 Residual standard deviation	4
5.2 Sensitivity of the analytical procedure	5
5.3 Standard deviation of the procedure	5
5.4 Relative standard deviation of the procedure	5
6 Analysis of a sample	5
6.1 General considerations	5
6.2 Test for minima or maxima	5
6.3 Calculation of the most probable concentration	6
6.4 Prediction interval of the analytical result (see figure 1)	6
7 Example	7
7.1 Calibration, performance characteristics, evaluation	7
7.2 Single measurement analysis	8
 Annexes	
A <i>F</i> -table (99 %)	10
B Bibliography	12

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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.

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.

International Standard ISO 8466-2 was prepared by Technical Committee ISO/TC 147, *Water quality*, Sub-Committee SC 7, *Precision and accuracy*.

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*
- *Part 3: Method of standard addition*
- *Part 4: Estimation of limit of detection and limit of determination of an analytical basis method*

Annex A forms an integral part of this part of ISO 8466. Annex B is for information only.

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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

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). Using this, 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

x_i	Concentration of the i th standard sample.
i	Subscript of the concentration levels, where $i = 1, 2, \dots, N$.
N	Number of concentration levels (for this part of ISO 8466, $N = 10$).
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).
$y_{i,j}$	j th information value for the concentration x_i .
j	Subscript of the replicates of level i , where $j = 1, 2, \dots, n_i$.
n_i	Number of replicates per level x_i .
\bar{y}_i	Mean of the information values $y_{i,j}$ of standard samples, having the concentration x_i .
s_i^2	Variance of the information values for the analyses of standard samples, having the concentration x_i .
PW	Test value for the F -test.
$F(f_1, f_2, P)$	Tabled value of the F -distribution with f_1 and f_2 degrees of freedom and a confidence level of P (%).
a, b, c	Coefficients of the calibration function.
\bar{x}	Mean of the standard concentrations x_i , resulting from the calibration experiment.

\bar{y}	Mean of the information values y_i , resulting from the calibration experiment.
\hat{y}_i	Information value of the standard concentration x_i calculated from the calibration function.
s_y	Residual standard deviation.
f	Degree of freedom for the residual standard deviation ($f = N - 3$).
e	Sensitivity = first derivative of the calibration function.
E	Sensitivity in the centre of the working range.
S_{x_0}	Standard deviation of the procedure.
V_{x_0}	Coefficient of variation of the procedure.
\hat{y}	Information value of an analytical sample.
\hat{x}	Concentration of the analytical sample, calculated from the information value \hat{y} .
\hat{N}	Number of replicates on the same analytical sample.
$VB(\hat{x})$	Confidence interval for the concentration \hat{x} .
$t(f_1, P)$	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).
x^*	Concentration at which the calibration function has minimum or maximum value.

3 Performance

3.1 Choice of working range

The calibrating experiment requires the establishment of a preliminary working range depending on

- a) the practice-related objective of the calibration.

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

- b) The values obtained near the lower limit of the working range being 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 determination 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 information values shall be homogeneous and independent of the concentration.

After the establishment of the preliminary working range, information values of at least $N = 5$ (recommended $N = 10$) standard samples are determined. The concentrations, x_i , of these standard samples shall be distributed equidistantly over the working range. In order to examine the homogeneity of the variances, n_i standards of each of the lowest and highest concentrations of the working range are measured as replicates. n_i information 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):

$$s_i^2 = \frac{\sum_{j=1}^{n_i} (y_{i,j} - \bar{y}_i)^2}{n_i - 1} \quad \dots (1)$$

where $f_i = n_i - 1$

with the mean

$$\bar{y}_i = \frac{\sum_{j=1}^{n_i} y_{i,j}}{n_i} \quad \dots (2)$$

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.

The test value PW required for the F -test is determined as follows:

$$PW = \frac{s_{10}^2}{s_1^2} \quad \text{for } s_{10}^2 > s_1^2 \quad \dots (3)$$

$$PW = \frac{s_1^2}{s_{10}^2} \quad \text{for } s_1^2 > s_{10}^2 \quad \dots (4)$$

PW is compared with the tabled values of the F -distribution (see annex A).

Decision:

- If $PW \leq F(f_1, f_2, 99 \%)$: the difference between the variances is not significant.
- If $PW > F(f_1, f_2, 99 \%)$: the difference between the variances is significant.

The preliminary working range in case b) should be decreased 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 information values y_i .

4 Estimation of the polynomial coefficients

Using the values of the standard concentrations as independent variables and the measured information values as dependent variables, calculate the calibration coefficients of the polynomial using equation (5)

$$y = a + bx + cx^2 \quad \dots (5)$$

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

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

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

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

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

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

Centre of the working range:

$$x = \frac{\sum x_i}{N} \quad \dots (11)$$

Average information value:

$$y = \frac{\sum y_i}{N} \quad \dots (12)$$

Estimation of the coefficients of the equation of the calibration function:

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

$$b = \frac{Q_{xy} - cQ_{x^3}}{Q_{xx}} \quad \dots (14)$$

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

In order to test the adequacy of the second order function, the residues $(y_i - \hat{y}_i)$ 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 information 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).

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

where

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

or

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

Degree of freedom:

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

5.2 Sensitivity of the analytical procedure

The sensitivity is derived from the change of the information 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 [1]. In the event of a non-linear calibration function, the sensitivity e is given by the first derivative of the calibration function as follows:

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

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

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

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

5.3 Standard deviation of the procedure

The standard deviation of the procedure s_{x_0} 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).

$$s_{x_0} = \frac{s_y}{E} \quad \dots (22)$$

The standard deviation of the procedure s_{x_0} (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 equally distributed within the working range.

5.4 Relative standard deviation of the procedure

The relative standard deviation of the procedure, V_{x_0} , allows a comparison of the performance of analytical procedures and is calculated, as a percentage, using equation (23).

$$V_{x_0} = \frac{s_{x_0} \times 100}{\bar{x}} \quad \dots (23)$$

6 Analysis of a sample

6.1 General considerations

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

The calibration function must 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 must not be used.

6.2 Test for minima or maxima

Using equation (20)

$$e = b + 2cx \text{ and}$$

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

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 most probable concentration

In order to obtain the target value concentration \hat{x} from the measuring value (information value) \hat{y} , the inverse function of equation (5) must be formed.

For positively curved calibration functions, apply equation (25):

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

For negatively curved calibration functions, apply equation (26):

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

6.4 Prediction 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 information value, but also of the error s_y , originating in the calibration function^[2].

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

- a) residual standard deviation s_y ;
- b) number N of standards used for the calibration;
- c) number \hat{N} of replicates 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 concentrations, $\hat{x} - \bar{x}$.

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

$$VB(\hat{x}) = \frac{s_y \cdot t_{1,P}}{(b + 2c\hat{x})} \cdot \left\{ \frac{1}{N} + \frac{1}{\hat{N}} + \frac{(\hat{x} - \bar{x})^2 Q_x^4 + \left(\hat{x}^2 - \frac{\sum x_i^2}{N}\right) Q_{xx} - 2(\hat{x} - \bar{x}) \left(\hat{x}^2 - \frac{\sum x_i^2}{N}\right) Q_x^3}{Q_x^4 Q_{xx} - (Q_x^3)^2} \right\}^{1/2} \quad \dots (27)$$

The analytical result is given by:

$$\hat{x}_{1,2} = \hat{x} \pm VB(\hat{x}) \quad \dots (28)$$

NOTE 1 In order to guard against rounding errors, it is recommended to use as many decimal places as possible in the calculation.

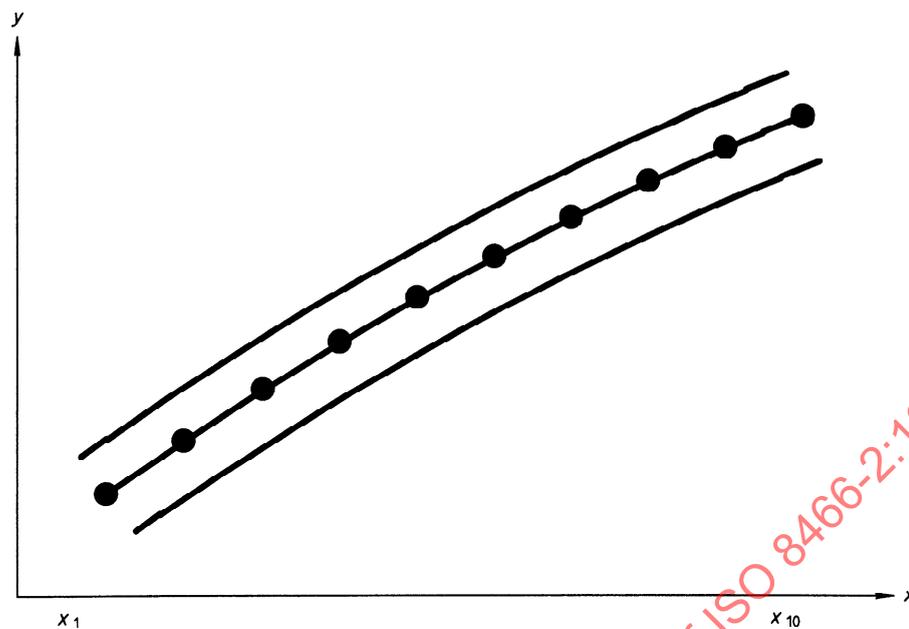


Figure 1 — Calibration function, second degree with prediction interval

7 Example

7.1 Calibration, performance characteristics, evaluation

Analytical procedure: theoretical example (see figure 2).

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

The homogeneity of the variances was proved by preliminary tests.

Calibration data (information values):

x_i mg/l	y_i (absorbance)
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 (absorbance)
b	-0,007 67 (absorbance, l/mg)
c	-0,000 02 (absorbance, l ² /mg ²)
s_y	-0,001 48 (absorbance)
s_{x_0}	-0,241 89 (mg/l à la concentration \bar{x})
V_{x_0}	0,6 (%)

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

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

$$x^* = -\frac{0,007\ 67}{2(-0,000\ 02)} = 191,7\ \text{mg/l}$$

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

7.2 Single measurement analysis

Information value of a sample:

$$\hat{y} = 0,084\ \text{(absorbance)}$$

Calculation of the analytical result, using equation (26):

$$\hat{x} = 12,1\ \text{(mg/l)}$$

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

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

The result is:

$$\hat{x}_{1,2} = (12,06 \pm 0,63) \text{ mg/l}$$

The true value of the unknown concentration is expected to be between 11,43 mg/l and 12,69 mg/l.

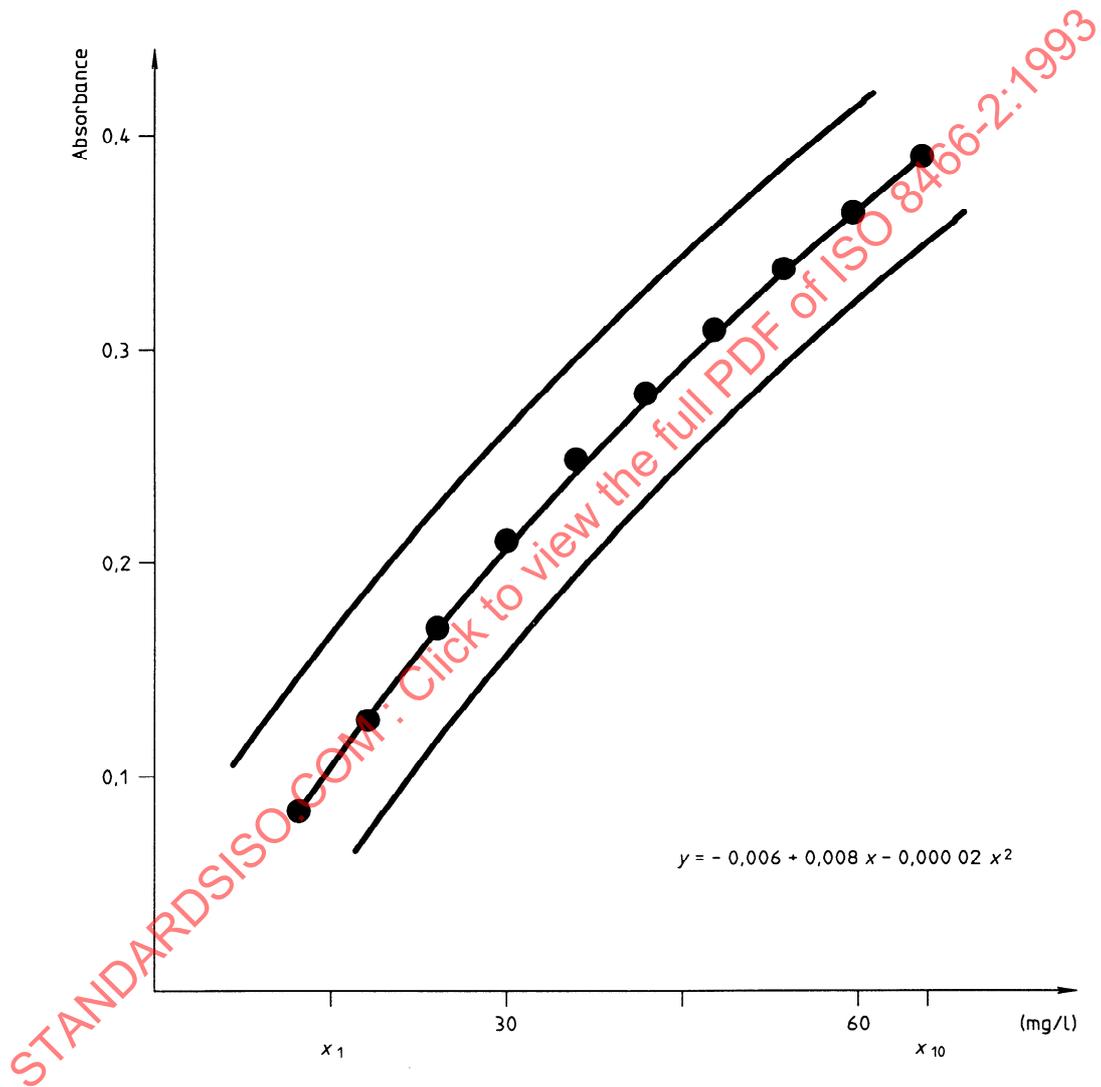


Figure 2 — Example of a calibration function of the second degree

Annex A
(normative)

***F*-table (99 %)**

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

<i>f</i> ₁ / <i>f</i> ₂	1	2	3	4	5	6	7	8	9	10	12	15	20	24	30	40	60	120	∞	
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	39,00	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	26,04
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	26,04
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	13,36
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	8,92
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	6,78
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	5,55
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	4,76
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	4,21
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	3,81
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	3,50
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	3,26
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	3,07
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	2,90
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	2,77
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	2,65
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	2,55
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	2,47
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	2,39
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	2,32
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	2,26
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	2,21
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	2,16
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	2,11
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	2,07
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	2,03
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	2,00
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	1,96
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	1,93
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	1,91
40	7,31	5,18	4,31	3,83	3,51	3,28	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	1,70
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	1,50
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	1,28
∞	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	0,90

Annex B
(informative)

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