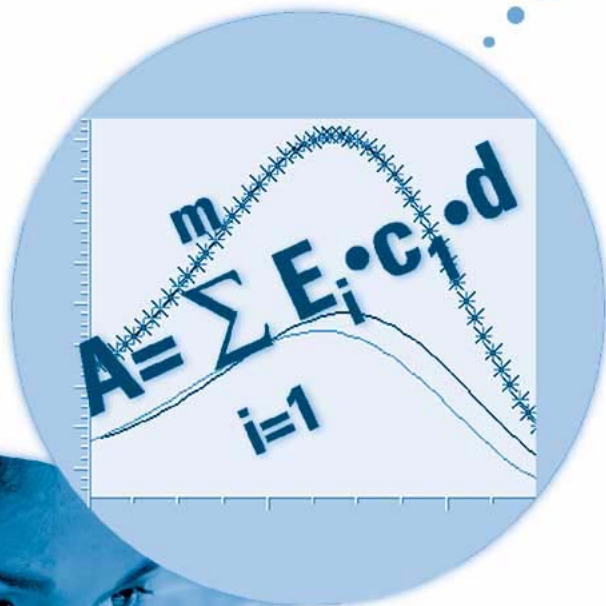




Agilent ChemStation for UV-visible Spectroscopy



**Understanding Your
Advanced Software**



Agilent Technologies

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Software Revision

This handbook is for B.02.01 revisions of the Agilent ChemStation software, where xx is a number from 00 through 99 and refers to minor revisions of the software that do not affect the technical accuracy of this handbook.

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In This Guide...

This manual describes the data processing operations of the advanced software for the Agilent ChemStation for UV-visible spectroscopy. It describes the processes and transformations that the spectrophotometric data undergo between the time that they are acquired in the spectrophotometer and their output on the Agilent ChemStation display or printer. The manual is designed to enable you to follow good laboratory practice (GLP) guidelines. Using the information in the manual, you will be able to understand the data processing calculations from beginning to end and perform the data evaluations manually.

1 Data Processing

This chapter explains the processes that take place when your acquired data are processed, so that you can understand in detail the data processing steps of the advanced software of the Agilent ChemStation.

2 Single Component Analysis (SCA)

This chapter describes the mathematical and statistical calculations that are used to produce a calibration curve for single component analysis and evaluate an unknown sample.

3 Multicomponent Analysis (MCA)

This chapter describes the mathematical and statistical calculations that are used to produce a calibration for multicomponent analysis and evaluate an unknown multicomponent sample.

4 Interactive Math Functions

This chapter contains full explanations of the operation of the mathematical functions, including their operation at limiting conditions.

5 Optimization

This chapter explains the operation of the optimization tasks available in the advanced software, and describes the calculations that are used and the importance of the results.

6 Automation

This chapter describes the processes of automatic sample measurement, spectral processing and evaluation in the advanced software.

7 Reports

This chapter explains the format and contents of the reports that are available in the advanced software.

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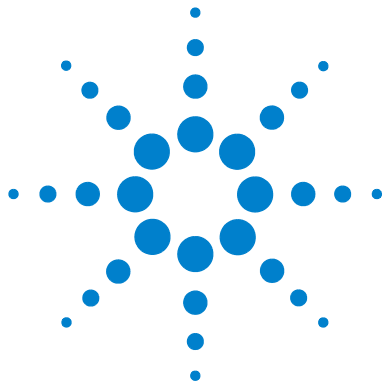
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1 Data Processing

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Spectral Processing

Sometimes, the analysis results may be improved by performing one or more mathematical operations on the raw spectral data before extracting the wavelength information. For example, noisy spectral data can be smoothed, and using derivatives instead of absorbance data can eliminate a constant baseline offset (first derivative) or a linearly increasing or decreasing baseline (second derivative). You can also reduce or eliminate the effects of light scattering, and interferences due to solvent and matrix effects. The spectral processing options are identical with the math processing operations, the details of which are fully explained in [Chapter 4](#), “Interactive Math Functions”.

Use Wavelengths

The diode-array detector of the spectrophotometer acquires spectral data at all wavelengths simultaneously—these data are available for the analysis of your sample. Instead of using the data at a single wavelength, you can therefore define several wavelengths or wavelength ranges for inclusion in the calculation of an **analytical function**. The result of the analytical function calculation is the **function result**. If you select a single wavelength, the function result is equal to the absorbance at that wavelength.

Analytical Wavelength

The analytical wavelength is the primary wavelength that you select for analyzing your sample. The analytical wavelength is typically the wavelength at the absorbance maximum, although the full-spectrum acquisition and virtually absolute wavelength reproducibility of the diode-array spectrophotometer allow you to select any wavelength in the absorbance band. If the maximum absorbance is high (for example, greater than 1 AU), selecting wavelengths at the side of an absorbance band, where the absorbance is lower, can avoid non-linearity due to stray light. Measurements on the side of absorbance bands are usually also included when measuring over a wavelength range, and in multicomponent analysis, when measurements over a wide spectral range are used.

Range of Wavelengths

Selecting a wavelength range instead of a single wavelength can improve sensitivity and reproducibility. As more data points are averaged together either side of the absorbance maximum, the average absorbance decreases slowly at first, then more quickly as low absorbances on the side of the band are included (see Figure 1 on page 12). At the same time as data points are averaged, noise decreases as the square root of the number of data points. Thus, the signal-to-noise ratio improves as more points are averaged, but eventually declines as more low absorbance values are included in the average. There is an optimum range which is generally equivalent to the bandwidth at the half-height of the absorbance band, that is the natural bandwidth (NBW).

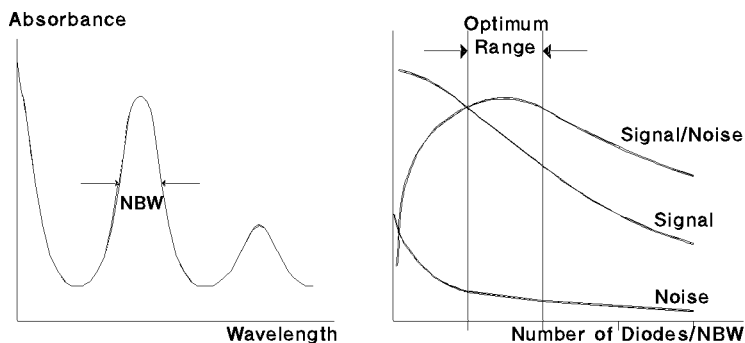


Figure 1 Optimum Signal-to-Noise Ratio

When more than one wavelength range is specified, the function result is the average of the average absorbance values of all specified ranges:

$$f = \frac{1}{n_A + \dots + n_N} \left(\sum_{i \in \lambda_{A_1} : \lambda_{A_n}} A_i + \dots + \sum_{i \in \lambda_{N_1} : \lambda_{N_n}} A_i \right) \quad (1)$$

where

$i \in \lambda_{A_1} : \lambda_{A_n}$ through $i \in \lambda_{N_1} : \lambda_{N_n}$ specify the analytical wavelength ranges

$N_A \dots N_N$ specify the number of data points within each range

For each wavelength range, the step specifies the interval between wavelength values for the calculation of the average absorbance. Where the step is different from the spectral resolution of the data, a linear interpolation is made where necessary. For example, if a step value of 3 nm is specified for data with a spectral resolution of 2 nm, a linear interpolation is made for every other point. Similarly, a linear interpolation is made for every point when the step value is the same as the spectral resolution of the data, but the starting wavelength of the range does not correspond exactly with an actual wavelength value. For multiple ranges, the specification of a smaller step value adds weight to the influence of the range in the function result.

Analytical Function

The Analytical Function option allows you to include internal referencing in the calculation of the function result. For details of internal referencing, see “[Internal Reference](#)” on page 59. The analytical function wavelength parameters include a factor, which is an alternative method for increasing or decreasing the influence of specific wavelength range in the function result. When the factor is not equal to 1, the average absorbance value of the wavelength range is multiplied by the factor before the function result is calculated.

$$f = \frac{1}{n_A + \dots + n_N} \left(\sum_{i \in \lambda_{A_1} : \lambda_{A_n}} F_A \cdot A_i + \dots + \sum_{i \in \lambda_{N_1} : \lambda_{N_n}} F_N \cdot A_i \right) \quad (2)$$

where

$i \in \lambda_{A_1} : \lambda_{A_n}$ through $i \in \lambda_{A_1} : \lambda_{A_n}$ specify the analytical wavelength ranges

$N_A \dots N_N$ specify the number of data points within each range

$F_A \dots F_N$ are the specified factors for the ranges

Evaluation

Advanced mode offers a choice of four evaluation procedures:

- None (no evaluation)
- Equation
- SCA (see [Chapter 2](#), “Single Component Analysis (SCA)”)
- MCA (see [Chapter 3](#), “Multicomponent Analysis (MCA)”)

When no evaluation is selected, the analysis results are the contents of the Wavelength Results register: the function result and the results from the individual wavelength(s).

Equation Evaluation

When an equation evaluation is specified, the analysis results are the results of up to four equations specified in the Equation Parameter dialog box. In the definition of the equations, you can use any result generated by the Use Wavelength(s) and stored in the Wavelength Results register (function result, results from individual wavelengths, average absorbance of a wavelength range) and any other variables that you have entered into the Sample Spectra table. For example, if you wish to use the weight of an analyte in an equation, you can add *weight* as an Analyte in the Sample Spectra table, and enter the appropriate value and units for the weight of the analyte. You can use the *weight* of the sample in the equation by defining *weight* as one of the four variables A1 to A4 in the Equation Parameter dialog box. When path length correction is switched on, it normalizes the results to a 1-cm path length by dividing the function result by the value of the Path Length parameter in the Sample Spectra table:

$$f_{\text{corr}} = \frac{f}{l} \quad (3)$$

where

f_{corr} is the function result after path length correction

f is the uncorrected function result

l is the path length

When dilution factor correction is switched on, the result is multiplied by the entry in the Sample Information dialog box:

$$f_{corr} = f \times d$$

where

f_{corr} is the function result after path length correction

f is the uncorrected function result

d is the dilution factor

Results from individual wavelengths or wavelength ranges are corrected for the path length and dilution factor in the same way as the function result.

Extended Analyses

By default, one data analysis is always available. The extended analysis facilities enable up to three additional data analyses to be defined in each method. The results of the additional data analyses can be simply collated and reported together, as multiple analyses, or can be used as confirmatory results in a confirmation analysis. In either case, the additional data analysis results can also be viewed independently.

Confirmation Analysis

Confirmation analysis uses additional analysis results as a check on the identity and purity of your samples, and whether the measurements are within the linear dynamic range of the instrument. Typically, this includes results at one or more additional analytical wavelengths called **confirmation wavelengths**, which are selected at various points on the sample spectrum, separate from the primary analytical wavelength but showing reasonable absorbance. Confirmation wavelengths are often chosen on either side of an absorbance band, generally at wavelengths as far away from the analytical wavelength as possible, but with a significant absorbance. Because it is unlikely that an impurity has the same spectrum as the analyte of interest, confirmation analysis gives a high probability of detecting unexpected impurities.

For example, for the analysis of pure caffeine, the analytical wavelength is set to 274 nm, the absorbance maximum. The absorbance measurements produce accurate results for caffeine when no impurities are present. If, however, there is a low level of salicylic acid impurity in the caffeine sample, the absorbance value measured at 274 nm is too high for the concentration of caffeine in the sample because of the additional absorbance due to salicylic acid (see Figure 2).

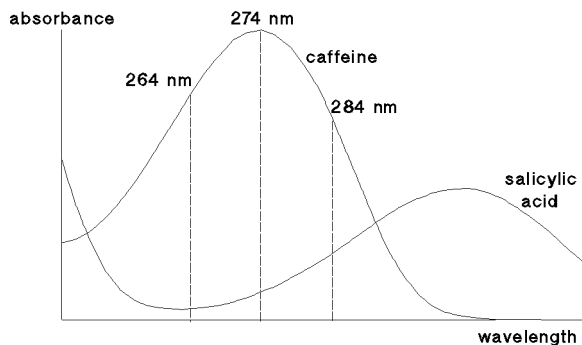
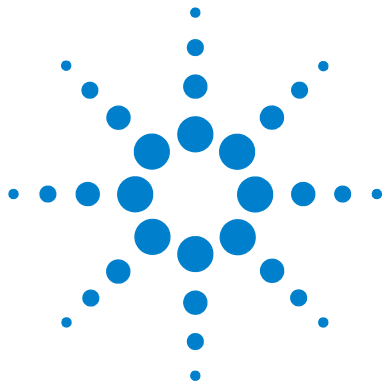


Figure 2 Confirmation Analysis

When two confirmation analyses are specified, using confirmation wavelengths at 264 nm and 284 nm, the confirmation results are different because the absorbance of salicylic acid relative to caffeine at each wavelength is different. The difference in the confirmation results is an immediate indication of the presence of an impurity.

In order for an analyte to be confirmed, the result of the confirmation analysis must be within a specified tolerance. If there are no impurities in the sample, the difference between the confirmation results and the analysis result will be within the specified tolerance. However, if there is an impurity in the sample that absorbs at one of the confirmation wavelengths, the difference between the confirmation result and the analysis result will be outside the tolerance, indicating the presence of the impurity.

1 Data Processing
Extended Analyses



2 Single Component Analysis (SCA)

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Overview

Two statistical methods are available for establishing the relationship between the measured data (for example, their absorbance) and the concentrations of a set of standards. It is always assumed that the variance in the measured data is less than the variance in the standard concentrations. The calibration curve graphs are shown in the more traditional way with the concentration modules (concentration times path length) on the x-axes and the measured data values on the y-axes.

Least Squares Method (LSQ)

The Least Squares Method is the default method. The calibration curve parameters are calculated to minimize the squares of the differences between the standard concentrations available with the set of standards and the calculated concentration points on the calibration curve. Under these conditions, the overall deviation of the calculated calibration curve from the standard concentrations is minimized.

Method of Maximum Likelihood (MLH)

The Method of Maximum Likelihood is a weighted calibration. The weights are the reciprocals of the squares of the estimated standard deviations of the residuals. These standard deviations are estimated from the standard deviations of the data and from standard deviations of the concentrations. So the influence of each point to the calibration is weighted according to his precision with respect to both its data value and its concentration (using the standard deviation of the analyte concentration from the Standard Spectra table). Instead of the minimization of the concentration difference, the weighted difference is minimized.

Beer-Lambert Law

The Beer-Lambert law states that the absorbance of a component in solution is directly proportional to its concentration:

$$A = Ecd \quad (4)$$

In [Equation 4](#):

A is absorbance

E is molar absorptivity or molar extinction coefficient ($\text{l mol}^{-1} \text{cm}^{-1}$)

c is analyte (component of interest) concentration (mol l^{-1})

d is cell path length (cm)

The concept of **analytical functions**, created by combining absorbance or derivative data from different parts of the spectrum to give a **function result**, f , means that these function results can be used instead of absorbance data in the following equations.

To calculate the concentration of an unknown compound, the above equation can be solved for a given function result, f :

$$c = \frac{f}{Ed} \quad (5)$$

The reciprocal of the product of molar extinction coefficient and cell path length is often called the calibration coefficient, k .

$$c = kf \quad (6)$$

SCA Calibration

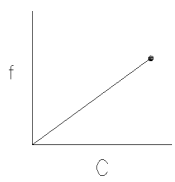
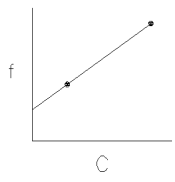
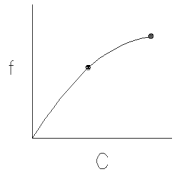
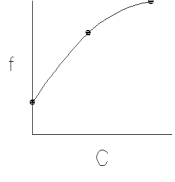
Calibration Curve Types

Real data may deviate slightly from the ideal linear relationship described in “[Beer-Lambert Law](#)” on page 21. The relationship between the function result and concentration may be described more accurately by adding an offset (a non-zero intercept) or a quadratic term (or both) to the equation.

The ChemStation provides four different calibration curve types, shown in [Table 1](#) on page 23. These calibration curve graphs are shown in the more traditional way with the concentration on the x-axis and the function results on the y-axis.

The Agilent ChemStation provides four different calibration curve types, shown in [Table 1](#).

Table 1 Calibration Curve Types for Single Component Analysis

Curve Type	Curve	Equation
I Linear without zero offset		$c = k_1 f$
II Linear with zero offset		$c = k_0 + k_1 f$
III Quadratic without zero offset		$c = k_1 f + k_2 f^2$
IV Quadratic with zero offset		$c = k_0 + k_1 f + k_2 f^2$

c is the concentration

f is the function result

k_0, k_1, k_2 are calibration coefficients

Number of Standards

There is no fixed limit to the number of calibration standards that can be incorporated into a calibration; the more points that can be placed on the curve, the more accurately the curve can be characterized. However, each of the calibration curve types requires a minimum number of standards of different concentrations (see [Table 2](#)).

Table 2 Minimum Number of Standards for Calibration Curves

Calibration Curve Type	Minimum Number of Standards
Linear, no zero offset (Beer's Law)	1
Linear with zero offset	2
Quadratic, no zero offset	2
Quadratic with zero offset	3

Calibration Standards

If the absorbance of the analyte at the wavelength(s) you select strictly obeys Beer's law, then one standard is sufficient to characterize the calibration curve (linear without zero offset). However, the use of two standards at different concentrations will confirm adherence to Beer's law, or show up any irregularities. Two standards are necessary to characterize a linear calibration curve with zero offset. Addition of a third standard at a different concentration is sufficient to identify and characterize a non-linear calibration curve.

Calibration Curve Fits

The mathematical problem is to find the calibration coefficients in a given curve type which will allow the best determination of a future unknown sample.

Least Squares Method

The least squares method uses the analytical function data from Use Wavelengths of all standards to determine the calibration coefficients of the chosen calibration curve type by a least squares calculation.

If each i^{th} data set (f_i, c_i) of the n standard data sets is expected to obey a function in the p coefficients, k_j , although the real (measured) values may cluster around the function because of statistical errors, ε_i , then the general equation is:

$$c_i = k_0 + k_1 f_i + k_2 f_i^2 + \varepsilon_i \quad (7)$$

where

i is 1, 2, 3, ... n (the total number of standards)

The calibration coefficients, k_j , can be estimated using the least squares method, that is minimizing the sum of the squares of the errors, ε_i (the differences between the measured value and the calibration curve).

$$\sum_{i=1}^n (c_{\text{actual}_i} - c_{\text{calculated}_i})^2 = \sum_{i=1}^n \varepsilon_i^2 = \text{minimum} \quad (8)$$

In matrix notation:

$$c = Fk + \varepsilon \quad (9)$$

where

c is n -concentration column vector

F is $n \times p$ function result data matrix

k is p -calibration coefficient column vector

ε is n -error column vector

The elements used in the calibration matrix F and the coefficient vector k are given in [Table 3](#) with dimension p of the coefficient vector.

Table 3 Elements Used in Calibration Matrix F

Curve Type	p	$F_{(i)\text{th row}}$			k		
I	1	f_i	—	—	k_1	—	—
II	2	1	f_i	—	k_0	k_1	—
III	2	f_i	f_i^2	—	k_1	k_2	—
IV	3	1	f_i	f_i^2	k_0	k_1	k_2

Example

Using curve type III, where $p = 2$, for 5 standards, $n = 5$:

$$c_1 = k_1 f_1 + k_2 f_1^2 + \varepsilon_1 \text{ through to } c_5 = k_1 f_5 + k_2 f_5^2 + \varepsilon_5$$

That is:

$$\begin{bmatrix} c_i \\ \dots \\ c_5 \end{bmatrix} = \begin{bmatrix} f_1 & f_1^2 \\ \dots & \dots \\ f_5 & f_5^2 \end{bmatrix} \begin{bmatrix} k_1 \\ k_2 \end{bmatrix} + \begin{bmatrix} \varepsilon_i \\ \dots \\ \varepsilon_5 \end{bmatrix} \quad \text{or, in matrix notation: } c = Fk + \varepsilon$$

By defining F and k for each of the calibration curve types, the coefficients are given by the general equation:

$$k = (F^T F)^{-1} F^T c \tag{10}$$

where

F^T denotes the transpose of F

Example continued:

$$\begin{bmatrix} k_1 \\ k_2 \end{bmatrix} = \left(\begin{bmatrix} f_1 & \dots & f_5 \\ f_1^2 & \dots & f_5^2 \end{bmatrix} \begin{bmatrix} f_1 & f_1^2 \\ \dots & \dots \\ f_5 & f_5^2 \end{bmatrix} \right)^{-1} \left(\begin{bmatrix} f_1 & \dots & f_5 \\ f_1^2 & \dots & f_5^2 \end{bmatrix} \begin{bmatrix} c_1 \\ \dots \\ c_5 \end{bmatrix} \right)$$

Method of Maximum Likelihood

In the maximum likelihood calculation, the standard deviations of the function results of the standards (calculated from the variances provided by the spectrophotometer) and the standard deviations of the concentrations (from the Analytes of Standards table) are used in a weighted calibration.

Now, instead of Equation 8 on page 25, the weighed sum of the squares of the residuals is minimized:

$$S_{MLH} = \sum_{i=1}^n w_i (c_{actual_i} - c_{calculated_i})^2 \quad (11)$$

The weights w_i are the reciprocals of the squares of the estimated standard deviations of the residuals:

$$w_i = \frac{1}{s_{\varepsilon_i}^2} \quad (12)$$

From Equation 7, it follows that ε_i depends on c_i and f_i , both of which are subject to error. Using the technique of error propagation, the standard deviations of ε_i can be estimated from the standard deviation of concentration, s_{c_i} , and the standard deviation of the function result, s_{f_i} , of the i^{th} standard.

$$s_{\varepsilon_i}^2 = s_{c_i}^2 + k_1^2 s_{f_i}^2 + 4k_1 k_2 f_i s_{f_i}^2 + 4k_2^2 f_i^2 s_{f_i}^2 \quad (13)$$

If maximum likelihood is specified, the least squares coefficients, k_1 and k_2 are used to evaluate $s_{\varepsilon_i}^2$ in Equation 13.

When S_{MLH} is minimized, the estimates, k , of the calibration coefficients can be obtained using Equation 14:

$$k = (F^T W F)^{-1} F^T W C \quad (14)$$

where the weighting matrix W is $diag(w_1, \dots, w_n)$ with w_i of Equation 12 on page 27.

SCA Calibration Results

This section contains the equations used in the calculations of the results given in the SCA Summary and the SCA Calibration Results table. For an explanation of the usage of the SCA Calibration Results, see [“Influential Standards and Outliers”](#) on page 35.

Residual of Concentration of i^{th} Standard

$$\varepsilon_i = c_{actual_i} - c_{calculated_i} \quad (15)$$

%Error

$$d_i = \frac{\varepsilon_i}{c_{calculated_i}} \times 100 \quad (16)$$

where

ε_i is the residual of the i^{th} standard, see [Equation 15](#).

Std.Dev of Calibration

Least Squares Method

$$s = \sqrt{\frac{1}{(n-p)} \sum_{i=1}^n \varepsilon_i^2} \quad (17)$$

where

ε_i is the residual of the i^{th} standard

n is the number of standards

p is the number of coefficients in the equation of the calibration curve

Maximum Likelihood

$$s_{sd} = \sqrt{\frac{n}{(n-p)} \cdot \frac{S_{MLH}}{n \sum_{i=1} w_i}} \quad (18)$$

The calculation for S_{MLH} is given in [Equation 11](#).

Relative Fit Error (Maximum Likelihood only)

$$e_{rf} = \frac{S_{MLH}}{n-p} \quad (19)$$

Std.Dev of Coefficient k_j

Least Squares Method

$$s_{k_j} = s \sqrt{(F^T F)^{-1}_{jj}} \quad (20)$$

where

$(F^T F)^{-1}_{jj}$ is the j^{th} diagonal element of $(F^T F)^{-1}$

s is the standard deviation of calibration, see [Equation 17](#).

Maximum Likelihood

$$s_{k_j} = s_{r_f} \sqrt{(F^T W F)^{-1}_{jj}} \quad (21)$$

where

j is $1, \dots, p$

Correl. Coeff. (R^2)

Least Squares Method

$$R^2 = \frac{\sum_{i=1}^n (c_{\text{calculated}_i} - \bar{c})^2}{\sum_{i=1}^n (c_{\text{actual}_i} - \bar{c})^2} \quad (22)$$

In the case of curve types II and IV,

$$\bar{c} = \frac{1}{n} \sum_{i=1}^n c_i \quad (23)$$

In the case of curve types I and III, \bar{c} is set to zero.

Maximum Likelihood

$$R^2 = \frac{\sum_{i=1}^n w_i (c_{\text{calculated}} - \bar{c})^2}{\sum_{i=1}^n w_i (c_{\text{actual}} - \bar{c})^2} \quad (24)$$

In the case of curve types II and IV,

$$\bar{c} = \frac{\sum_{i=1}^n w_i c_i}{\sum_{i=1}^n w_i} \quad (25)$$

The calculation of w_i is given in [Equation 12](#).

In the case of curve types I and III, \bar{c} is set to zero.

Leverage of the i^{th} Standard

Least Squares Method

$$h_i = (F(F^T F)^{-1} F^T)_{ii} \quad (26)$$

Maximum Likelihood

$$h_i = (W^{1/2} F (F^T W F)^{-1} F^T W^{1/2})_{ii} \quad (27)$$

99% CI

Least Squares Method

$$CI_i = t_{n-p, 99} \cdot s \cdot \sqrt{h_i} \quad (28)$$

where

$t_{n-p, 99}$ is the percentage point of the t distribution

s is the standard deviation of calibration, see [Equation 17](#)

h_i is the leverage of the i^{th} standard, see [Equation 26](#)

Maximum Likelihood

$$CI_i = t_{n-p, 99} \cdot \sqrt{e_{rf}} \cdot \sqrt{F(F^T W F)^{-1} F^T} \quad (29)$$

where

$t_{n-p, 99}$ is the percentage point of the t distribution

e_{rf} is the relative fit error, see [Equation 19](#)

Uncertainty

$$Uncertainty = \frac{PI_{i_{max}}}{c_{\text{calculated}_{i_{max}}}} \times 100 \quad (30)$$

where

$PI_{i_{max}}$ is the 95% Prediction Interval, see [Equation 38](#), of the standard with the highest absolute value of function result, that is, f_{unk} is set to $\max(f_i)$.

Stud.Res. (Studentized Residual)

Least Squares Method

$$t_i = \frac{\varepsilon_i}{s\sqrt{1-h_i}} \quad (31)$$

where

ε_i is the residual of the i^{th} standard, see [Equation 15](#)

s is the standard deviation of calibration, see [Equation 17](#)

h_i is the leverage of the i^{th} standard, see [Equation 26](#)

Maximum Likelihood

$$t_i = \frac{\sqrt{w_i} \cdot \varepsilon_i}{\sqrt{e_{rf}} \cdot \sqrt{1-h_i}} \quad (32)$$

where

ε_i is the residual of the i^{th} standard, see [Equation 15](#)

e_{rf} is the relative fit error, see [Equation 19](#)

h_i is the leverage of the i^{th} standard, see [Equation 27](#)

w_i is the weight of the i^{th} standard, see [Equation 12](#)

Cook's Dist. (Cook's Distance)

$$CD_i = \frac{t_i^2}{p} \cdot \frac{h_i}{1-h_i} \quad (33)$$

where

- t_i is the studentized residual of the i^{th} standard
(see “[Stud.Res. \(Studentized Residual\)](#)” on page 33),
depending on LSQ or MLH
- h_i is the leverage of the i^{th} standard
(see “[Leverage of the \$i^{\text{th}}\$ Standard](#)” on page 31),
depending on LSQ or MLH
- p is the number of coefficients

Influential Standards and Outliers

This section uses three sets of data to illustrate the interpretation of the SCA Calibration Results:

- Data Set 1 contains six points that fit to a type I calibration curve.
- In Data Set 2, the sixth point is replaced by an outlier of lower value.
- In Data Set 3, the third point is replaced by an outlier of lower value.

The calibration curves for the three sets of data are shown in [Figure 3](#) and the contents of the SCA Calibration Results tables are given in [Table 4](#) on page 36.

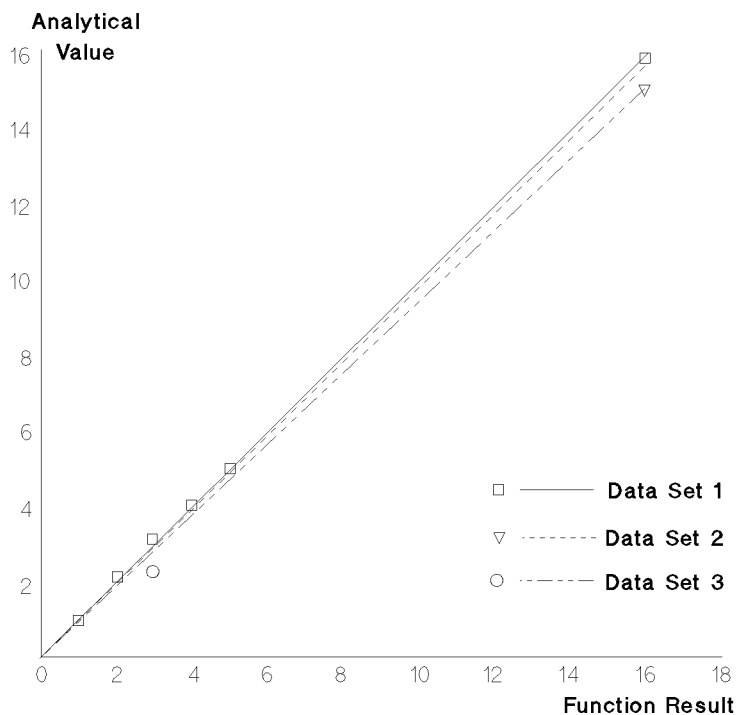


Figure 3 Calibration Curves of the Example Data Sets

2 Single Component Analysis (SCA)

Influential Standards and Outliers

Table 4 SCA Calibration Results for the Example Data Sets

Function Result	Value	Calculated Value	Residual	% Error	95% CI	Leverage	Stud. Res.	Cook's Distance
Data Set 1								
1	0.90	1.020	-0.12	-11.77	0.241	0.282	-1.434	0.403
2	2.10	2.010	0.09	4.48	0.220	0.233	1.041	0.165
3	3.10	3.000	0.10	3.33	0.202	0.198	1.131	0.158
4	4.00	3.990	0.01	0.25	0.191	0.176	0.112	0.001
5	4.90	4.980	-0.08	-1.61	0.186	0.167	-0.888	0.079
16	15.87	15.870	-1.8e-15	-1.1e-14	0.442	0.945	-7.7e-14	5.0e-26
Data Set 2								
1	0.90	1.135	-0.235	-20.73	0.347	0.282	-1.954	0.749
2	2.10	2.063	0.037	1.80	0.315	0.233	0.298	0.014
3	3.10	2.990	0.110	3.67	0.291	0.198	0.861	0.091
4	4.00	3.918	0.082	2.10	0.274	0.176	0.636	0.043
5	4.90	4.845	0.055	1.13	0.267	0.167	0.421	0.018
16	15.00	15.048	-0.048	-0.32	0.636	0.945	-1.439	17.696
Data Set 3								
1	0.90	0.816	0.084	10.28	0.872	0.282	0.277	0.015
2	2.10	1.819	0.281	15.44	0.793	0.233	0.900	0.123
3	2.20	2.822	-0.622	-22.04	0.730	0.198	1.947	0.467
4	4.00	3.825	0.175	4.58	0.688	0.176	0.541	0.031
5	4.90	4.828	0.072	1.49	0.671	0.167	0.222	0.005
16	15.87	15.860	0.010	0.06	1.596	0.945	0.119	0.120

An outlier is a standard that has a greater influence on the calculated calibration coefficients than the other standards in the data set. Standards that do not fit to the calibration model may often be obvious from the calibration plot. In Data Set 2, the sixth standard is an outlier; in Data Set 3, the third standard is an outlier. An outlier can be identified, or its influence estimated by deleting it from the data set and examining the differences in the calibration results.

The reported Leverage value indicates the potential influence of a standard on the calibration results. Leverage is dependent on the function result, but independent of the analyte value; this is obvious from the three example data sets, which have identical Leverage values, even though their analyte values are different. The value of Leverage lies in the range from $1/n$ to 1 (where n is the number of standards); the closer to 1 the Leverage is, the greater the probable influence of the standard. The sixth standard in the example data sets has a high leverage (0.945), and therefore has a potentially high influence on the calibration results

It can be shown that standards with high Leverage values are likely to have small Residuals. To counter this, the Residual is scaled by an expression that includes the Leverage to give the Studentized Residual. The Studentized Residual lies in a range between $-(n-p)^{1/2}$ and $(n-p)^{1/2}$ (p is the number of calculated calibration coefficients). The third standard of Data Set 3 is obviously an outlier; its Studentized Residual is more than twice the value of that of the other standards.

Cook's Distance is a measure of the actual influence of a standard. The outlier of Data Set 3 (the third standard) has less influence on the calibration curve than the outlier of Data Set 2. This is because of the difference in Leverage of the two outliers; the impact on the calibration curve is shown by the respective Cook's Distances (0.467 compared with 17.696). Deletion of a standard with a large Cook's Distance can result in dramatic improvements in the calibration results.

SCA Evaluation

When the standards have been measured, and the calibration coefficients have been determined, the unknown concentration of a measured sample can be calculated simply by calculating c_{unk} in the equation of the calibration curve using the function result data, f_{unk} of the unknown sample:

$$c_{unk} = k_0 + k_1 f_{unk} + k_2 f_{unk}^2 \quad (34)$$

where

k_0 ,

k_1 or

k_2 may be zero depending on the calibration curve type (see [Table 1](#)).

SCA Quantification Results

Std.Dev.

Least Squares Method

$$s_{c_{unk}} = s \sqrt{f_{unk} (F^T F)^{-1} f_{unk}^T} \quad (35)$$

where

s is the standard deviation of calibration, see [Equation 17](#)

f_{unk} is (f_{unk}) for calibration curve type I

or $(1, f_{unk})$ for calibration curve type II

or (f_{unk}, f_{unk}^2) for calibration curve type III

or $(1, f_{unk}, f_{unk}^2)$ for calibration curve type IV

F is the calibration matrix

Maximum Likelihood

$$s_{c_{unk}} = s_{rf} \sqrt{f_{unk} (F^T W F)^{-1} f_{unk}^T} \quad (36)$$

95% PI

Least Squares Method

$$PI = t_{n-p, 95} \cdot s \sqrt{f_{unk} (F^T F)^{-1} f_{unk}^T + 1} \quad (37)$$

where

$t_{n-p, 95}$ is the percentage point of the t distribution

s is the standard deviation of calibration, see [Equation 17](#)

f_{unk} and

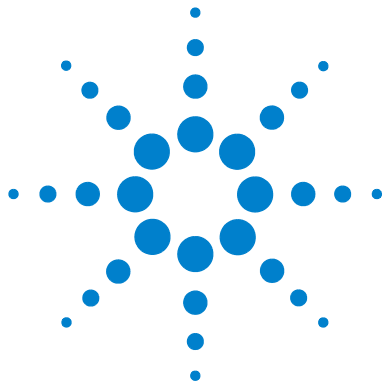
F are the same as for the standard deviation, see [Equation 35](#)

Maximum Likelihood

$$PI = t_{n-p, 95} \cdot s_{rf} \sqrt{f_{unk} (F^T W F)^{-1} f_{unk}^T + s_{unk}^2} \quad (38)$$

s_{unk} is calculated from [Equation 13](#), setting $s_{c_i}^2$ to zero and f_i equal

to f_{unk} , $s_{f_i}^2$ equal to $s_{f_{unk}}^2$.



3 Multicomponent Analysis (MCA)

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Overview

Multicomponent analysis is based on an extension of Beer's law to m components:

$$A = \sum_{i=1}^m E_i c_i d \quad (39)$$

where

A is the absorbance of a mixture of m components

E_i is molar absorptivity or molar extinction coefficient of the i^{th} component ($\text{l mol}^{-1} \text{ cm}^{-1}$)

c_i is the concentration of the i^{th} component (mol l^{-1})

d is cell path length (cm)

Equation 39 is applied at each wavelength. As with single component analysis (see Chapter 2, "Single Component Analysis (SCA)"), the multicomponent calibration uses the analytical function concept of combining absorbance or derivative data to give function results. However, instead of calculating the analytical function as an average, as in single component analysis, multicomponent analysis calculates individual function results for each wavelength.

The basic assumption in multicomponent analysis is that [Equation 39](#)—Beer’s law—applies at each wavelength except for a statistical error brought about by the use of real (measured) values.

$$f_j = \sum_{i=1}^m E_{ji}c_i d + \varepsilon_j \quad j = 1, \dots, n \quad (40)$$

where

m is the number of components

n is the number of wavelengths

f_j is the function result at the j^{th} wavelength

E_{ij} is molar absorptivity or molar extinction coefficient
 of the i^{th} component at the j^{th} wavelength

ε_j is the statistical error at wavelength j

Calculation Methods

In the multicomponent analysis calibration, the n times m individual molar absorptivity constants are calculated from standards, which may be either pure components or mixtures of the m components at known concentrations.

The calibration of component behavior by the standards can be formulated as follows:

$$f_{jk} = \sum_{i=1}^m E_{ji}c_{ik}d + \varepsilon_{jk} \quad j = 1, \dots, n \quad k = 1, \dots, p \quad (41)$$

where

p is the number of standards

c_{ik} is the concentration of the i^{th} component of the k^{th} standard; $c_{ik} = 0$, if the k^{th} standard has no i^{th} component

ε_{jk} is the statistical error of the k^{th} standard at wavelength j

A more compact form of Equation 41 is given by the matrix notation:

$$F = HC + \varepsilon \quad (42)$$

where

H is called the calibration coefficient matrix; it consists of $E_{ji}d$

C is the standard concentration matrix;
the elements are the products of d and C_{ik}

As with single component analysis (see Chapter 2, “Single Component Analysis (SCA)”), two calculation methods (least squares and maximum likelihood) are offered for the determination of the coefficient matrix H .

Least Squares

The least squares solution of Equation 42 is given by Equation 43:

$$H = FC^T(CC^T)^{-1} \quad (43)$$

Maximum Likelihood

In the case of maximum likelihood, the standard deviations, s_{jk} of the statistical errors ε_{jk} are estimated by error propagation from the standard deviations of the function results, $s_{F_{jk}}$, and the standard deviations of the concentrations entered in the Analytes of Standard table, $s_{C_{ik}}$.

$$s_{jk}^2 = s_{F_{jk}}^2 + \sum_{i=1}^m h_{ji}^2 s_{C_{ik}}^2 \quad (44)$$

For each wavelength, j , we define a p by p weighting matrix,

$$\phi_j = \text{diag}(s_{jk}^2) \quad (45)$$

and use it to calculate the j^{th} row of the calibration coefficient matrix, H , from the j^{th} row of the standard matrix, F , by a weighted least squares method.

$$H_j = F_j \phi_j^{-1} C^T (C \phi_j^{-1} C^T)^{-1} \quad (46)$$

where

H_j is the j^{th} row of H

F_j is the j^{th} row of F

The maximum likelihood method works in two steps:

First, the matrix H is estimated by an ordinary least squares method. The weighting matrixes, ϕ_j , are calculated for each wavelength, and the final matrix, H , is calculated row by row by a weighted least squares method.

Finally, the standard deviations of the calibration coefficients H_{ji} are calculated and stored for use in the analysis of the unknown samples.

$$s_{H_{ji}}^2 = \sum_{k=1}^p s_{F_{jk}}^2 (\phi_{jkk}^{-1} C_{ki}^+) \quad (47)$$

where

C_{ki}^+ is the individual element of the matrix product $C^T (C \phi_j^{-1} C^T)^{-1}$

MCA Calibration Results

Ind. of Stds, Std.Dev.Residual and Rel.Fit Error

At the end of the calibration process, the calibration standards are evaluated as unknowns (“MCA Evaluation” on page 48), and the standard deviation of the residual is calculated using Equation 55 (least squares) or Equation 56 (maximum likelihood) in “Std.Dev.Residual” on page 51. The independence of standards is also calculated using Equation 50 on page 49 in “Ind.of Stds” on page 50. In the case of the maximum likelihood method, the relative fit error is also calculated, using Equation 57 in “Rel.Fit Error” on page 51.

The Statistical Approach

The parameters estimated by the ordinary least squares in Equation 43 on page 44 can be shown to be “efficient” or “best” (that is, with minimum variance) if the following assumptions are true:

- 1 The regression function, Equation 42 on page 44, is correct. In multicomponent analysis, this means that Beer’s law is obeyed without offset and without chemical or instrumental (detector) non-linearity. For calibrations using pure standards, it also means that no interactions between the standards occur when they are in admixture that alter the absorbance. Equation (3.4) also implies that the mixture contains no absorbing compounds other than those in the standards.
- 2 Columns in matrix F are linearly independent, that is, the standard spectra are sufficiently different that $F^T F$ is non-singular.
- 3 F is known, that is, F is error-free, or has considerably smaller errors than those of C .

- 4 The errors of random variables C are stochastically independent.
- 5 Errors have normal distribution, with zero mean errors. This means that any systematic error must be negligible; ideally, there should be no systematic error. Systematic errors can arise from distortion of standard or sample spectra due to light scattering, stray light and other effects.
- 6 Errors have constant variance, that is, there is no difference in expected noise of data at different wavelengths.

Integrity of the Analysis

When mathematical mechanisms are applied to real-life data, there are always some assumptions that are not rigorously true. The computed results are never mathematically exact. In the quantification software, the assumptions 1, 5 and 6 in “[The Statistical Approach](#)” on page 46 are the most important causes of error; the maximum likelihood method addresses assumption 6. The best way to verify the integrity of the multicomponent analysis is to test it with known samples to ensure that the errors due to the violation of various assumptions are negligible relative to the required accuracy.

MCA Evaluation

The concentrations of an unknown mixture of the calibrated components can be predicted by solving [Equation 42](#) on page 44 in “[Calculation Methods](#)” on page 44, but now with known coefficients and unknown concentrations.

$$F_{unk} = HC_{unk} + \varepsilon_{unk} \quad (48)$$

where

F_{unk} is an n by 1 matrix of the function results of the sample

C_{unk} is an m by 1 matrix of the component concentrations of the sample

ε_{unk} is an n by 1 matrix of the statistical errors

Least Squares

The least squares solution of [Equation 48](#) is given by [Equation 49](#):

$$C_{unk} = (H^T H)^{-1} H^T F_{unk} \quad (49)$$

Maximum Likelihood

In the case of the maximum likelihood calculation, a weighting matrix, Ω , is calculated from the standard deviations of the calibration coefficients, $s_{H_{ji}}$, and the standard deviations of the function results of the sample, $s_{F_{unk_j}}$.

$$\Omega_{jj} = s_{F_{unk_j}}^2 + \sum_{i=1}^m s_{H_{ji}}^2 C_{unk_i}^2 \quad (50)$$

where

C_{unk_i} is the least squares estimate of the unknown sample concentrations

The final maximum likelihood estimates of the unknown concentrations are given by the weighted least squares method, with Ω as the weighting matrix:

$$C_{unk} = (H^T \Omega^{-1} H)^{-1} H^T \Omega^{-1} F_{unk} \quad (51)$$

where $\Omega = \text{diag}(\Omega_{11}, \dots, \Omega_{nn})$

MCA Quantification Results

Ind.of Stds

To characterize the calibration and evaluation, the independence of standards (*ios*) is calculated. For the least squares method, the independence of standards is calculated using [Equation 52](#):

$$ios = \frac{trace(H^T H) \cdot trace((H^T H)^{-1})}{m^2} \quad (52)$$

where

trace() is the sum of the diagonal elements of a square matrix

In the case of maximum likelihood, the independence of standards is calculated using [Equation 53](#):

$$ios = \frac{trace(H^T \Omega^{-1} H) \cdot trace((H^T \Omega^{-1} H)^{-1})}{m^2} \quad (53)$$

Function Result Residual

To verify the quality of the analysis, the function result residual is calculated from the concentration results, C_{unk} , the calibration coefficient matrix, H , and the sample function results, F_{unk} :

$$e_{unk} = F_{unk} - HC_{unk} \quad (54)$$

Std.Dev.Residual

In the case of the least squares method, the standard deviation of residual, s_{res} , is given by Equation 55:

$$s_{res} = \sqrt{\frac{1}{n-m} e_{unk}^T e_{unk}} = \sqrt{\frac{1}{n-m} \sum_{j=1}^n e_{unk_j}^2} \quad (55)$$

In the case of maximum likelihood, the standard deviation of residual is given by Equation 56:

$$s_{res} = \sqrt{\frac{\sum_{j=1}^n \frac{1}{\Omega_{jj}} \cdot e_{unk_j}^2}{\frac{n}{n-m} \cdot \frac{n}{\sum_{j=1}^n \frac{1}{\Omega_{jj}}}}} \quad (56)$$

Rel.Fit Error

The relative fit error, e_{rf} , is calculated for the method of maximum likelihood by Equation 57:

$$e_{rf} = \frac{1}{n-m} e_{unk}^T \Omega^{-1} e_{unk} = \frac{1}{n-m} \sum_{j=1}^n \frac{1}{\Omega_{jj}} e_{unk_j}^2 \quad (57)$$

Standard Deviations of Concentration

The standard deviations of the resulting concentrations are calculated using [Equation 58](#) in the case of the least squares calculation and [Equation 59](#) for the method of maximum likelihood.

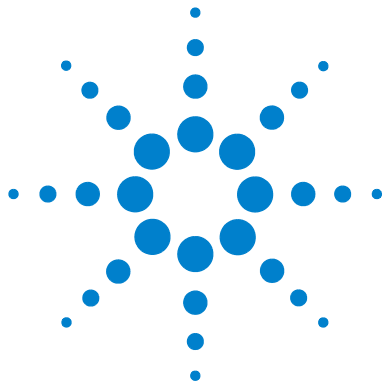
$$s_{C_{unk_i}} = \sqrt{s_{res}^2 (H^T H)^{-1}_{ii}} \quad i = 1, \dots, m \quad (58)$$

$$s_{C_{unk_i}} = \sqrt{s_{rf}^2 (H^T \Omega^{-1} H)^{-1}_{ii} + (C \phi^{-1} C^T)^{-1}_{ii} C_{unk_i}^2} \quad (59)$$

In [Equation 59](#),

C is the calibration concentration matrix

ϕ is the element-wise average of all weighting matrixes ϕ_j of [Equation 45](#) in “[Maximum Likelihood](#)” on page 45.



4 Interactive Math Functions

Unitary Operations 54

Binary Operations 70

NOTE

The action of the mathematical functions in interactive mode (using the Math menu commands) and in the Spectral Processing section of data analysis (see [Chapter 1](#), “Data Processing”) is identical.



Unitary Operations

The unitary mathematical functions operate on single spectra. If more than one spectrum is selected, the function operates on all selected spectra individually. If no spectra are selected, the operation is aborted and an error message is displayed.

Absorbance

Absorbance is the default method of spectral storage in the UV-visible ChemStation. The Absorbance function uses the Logarithm and Scalar Multiply functions to transform transmittance spectra into absorbance spectra using [Equation 60](#). If the data are already absorbance, no processing is performed.

$$A = -\log_{10}\left(\frac{T}{100}\right) \quad (60)$$

where

A is absorbance

T is transmittance in percent

The variances of the spectrum (if available) are transformed by [Equation 61](#):

$$\text{var}(A) = \left(\frac{1}{T \cdot \ln 10}\right)^2 \text{var}(T) \quad (61)$$

The availability of variance data is dependent on the condition of the Std. Deviation On check box in the Spectrophotometer Parameter dialog box, and on the integration time, also set in the Spectrophotometer Parameter dialog box. Variance data are not available for integration times of less than 0.2 seconds, and are not transferred when the Std. Deviation On check box is cleared.

Derivative

The derivative function calculates the derivative of the data points (y-values) in the spectrum using a “Savitsky-Golay Algorithm” on page 55 and places the resulting spectrum in the math result register. The underlying function is used for both derivative and smoothing (see “Derivative” on page 55 and “Smooth” on page 66).

Savitsky-Golay Algorithm

The Savitsky-Golay algorithm uses the Derivative Order, Filter Length and Polynomial Degree from the Derivative Parameter dialog box. For each data point in the spectrum, the calculation takes a set of data points equal to the filter length around the current data point, fits a curve of the specified polynomial degree, using a least squares fit. The fitted curve is then used to calculate the new value for the current data point, and the derivative of that point.

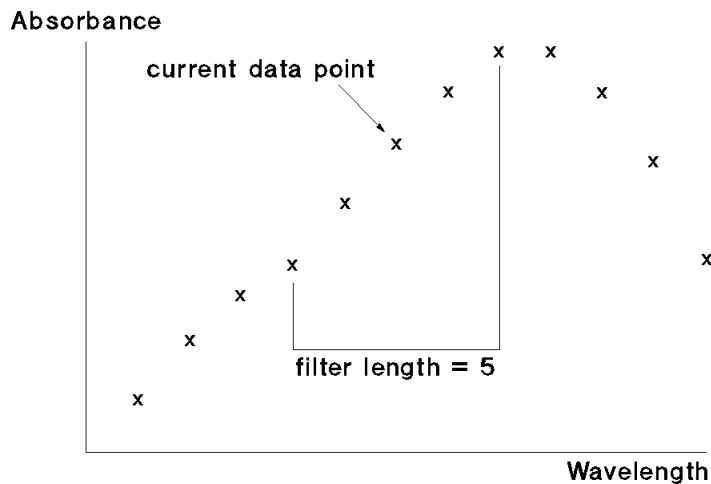


Figure 4 Filter Length in the Savitsky-Golay Algorithm

4 Interactive Math Functions

Unitary Operations

For each y-value, y_i ,

$$\text{deriv}(y_i) = \sum C_{kj} \cdot y_{i+j-1} \quad (62)$$

where

i is 1 ... data points-(filter length-1)

j is 1 ... filter length

k is 1 for smoothing, 2 for derivative

$\text{deriv}(y_1)$ corresponds to the $\left(\frac{(\text{filter length}-1)}{2} + 1\right)$ th value. For example, if *filter length* is 5, then $\text{deriv}(y_1)$ becomes y_3 . For this reason, the length of the spectrum is reduced by $(\text{filter length} - 1)$ values; $\left(\frac{(\text{filter length}-1)}{2}\right)$ values at the beginning of the spectrum and $\left(\frac{(\text{filter length}-1)}{2}\right)$ values at the end of the spectrum are not processed.

In [Equation 62](#) on page 56, C is the coefficient matrix:

$$C = N^{-1} \cdot F^T \quad (63)$$

where

N^{-1} is the inverse of N , the product of F^T and F

F^T is the transpose of F , the matrix of the powers of the polynomials

The matrix of the powers of the polynomials is generated using [Equation 64](#):

$$F_{ij} = k^{(j-1)} \quad (64)$$

where

i is 1 ... filter length

j is 1 ... degree +1

k is $i - \frac{\text{filter length} - 1}{2} - 1$

In the case of Derivative, the y-values are multiplied by the reciprocal of the step at the end of the calculation:

$$y_i = \frac{y_i}{\text{step}} \quad (65)$$

where

step is the increment of the equidistant x-axis

For each variance value, $\text{var}(y_i)$,

$$\text{deriv}(\text{var}(y_i)) = \sum C_{kj}^2 \cdot \text{var}(y_{i+j-1}) \quad (66)$$

where

C is the coefficient matrix

i is **1 ... data points - (filter length -1)**

j is **1 ... filter length**

k is 1 for smoothing, 2 for derivative

In the case of Derivative, the variance values are multiplied by the square of the reciprocal of the step at the end of the calculation:

$$\text{var}(y_i) = \frac{\text{var}(y_i)}{\text{step}^2} \quad (67)$$

If one of the values needed for the calculation is invalid, the resulting y-value or variance value is invalid.

All additional information, such as annotations, are preserved unchanged in the derivative spectrum.

Exponential

The exponential function calculates the exponential of each of the data points (y-values) in the spectrum and places the resulting spectrum in the math result register.

$$y_{\text{exp}} = e^y \quad (68)$$

The variances of the exponential spectrum, if available, are calculated according to [Equation 69](#):

$$\text{var}(y_{\text{exp}}) = e^{2y} \cdot \text{var}(y) \quad (69)$$

where

y is the original y-value

All additional information, such as annotations, are preserved unchanged in the exponential spectrum.

Internal Reference

Internal referencing is a technique that can be used to improve the precision of results by minimizing the effects of any changes that cause a linear baseline shift, for example a drift in lamp intensity. Internal referencing is particularly useful for samples with low absorbance.

The internal reference function allows the correction to be made by subtraction, multiplication or division.

Subtract

If the baseline shift is the same at all wavelengths, then it can be eliminated by subtracting the absorbance at the reference wavelength from the absorbance at the analytical wavelength, as in [Figure 5](#).

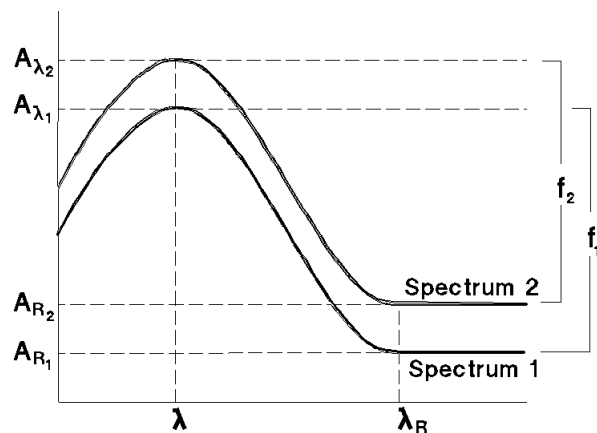


Figure 5 Internal Referencing

4 Interactive Math Functions

Unitary Operations

The reference wavelength is usually selected at a point on the baseline beyond the sample absorbance.

Subtraction is carried out using [Equation 70](#):

$$f_{\lambda} = A_{\lambda} - A_{R_{\lambda}} \quad (70)$$

where

f_{λ} is the function result at wavelength λ

A_{λ} is the absorbance at wavelength λ

$A_{R_{\lambda}}$ is the absorbance at reference wavelength λ

The variances (if available) are treated according to [Equation 71](#):

$$\text{var}(f_{\lambda}) = \text{var}(A_{\lambda}) + \text{var}(A_{R_{\lambda}}) \quad (71)$$

where

$\text{var}(f_{\lambda})$ is the variance of the function result at wavelength λ

$\text{var}(A_{\lambda})$ is the variance of the absorbance at wavelength λ

$\text{var}(A_{R_{\lambda}})$ is the variance of the absorbance at reference wavelength λ

Multiply

Multiplication is carried out using [Equation 72](#):

$$f_{\lambda} = A_{\lambda} \cdot A_{R_{\lambda}} \quad (72)$$

where the terms are the same as for subtraction (see [“Subtract”](#) on page 74).

The variances (if available) are treated according to [Equation 73](#):

$$\text{var}(f_{\lambda}) = A_{\lambda}^2 \cdot \text{var}(A_{R_{\lambda}}) + A_{R_{\lambda}}^2 \cdot \text{var}(A_{\lambda}) \quad (73)$$

where the terms are the same as for subtraction (see [“Subtract”](#) on page 74).

Divide

Division is carried out using [Equation 74](#):

$$f_{\lambda} = \frac{A_{\lambda}}{A_{R_{\lambda}}} \quad (74)$$

where the terms are the same as for subtraction (see “[Subtract](#)” on page 74).

The variances (if available) are treated according to [Equation 75](#):

$$\text{var}(f_{\lambda}) = \frac{1}{A_{R_{\lambda}}^2} \cdot \text{var}(A_{\lambda}) + \frac{A_{\lambda}^2}{A_{R_{\lambda}}^4} \cdot \text{var}(A_{R_{\lambda}}) \quad (75)$$

where the terms are the same as for subtraction (see “[Subtract](#)” on page 74).

Single Reference Wavelength or Wavelength Range

If the reference wavelength table contains only one row, then the absorbance at the reference wavelength, $A_{R_{\lambda}}$ is the average of the absorbance values taken from the specified wavelength range. This means that both $A_{R_{\lambda}}$ and $\text{var}(A_{R_{\lambda}})$ are constant.

Two Reference Wavelengths or Wavelength Ranges

If the reference wavelength table contains two rows, then the average of the absorbance and variance values from both reference wavelength ranges are taken, giving A_{R_1} , A_{R_2} , $\text{var}(A_{R_1})$ and $\text{var}(A_{R_2})$. The average wavelengths of the two wavelength ranges, λ_{R_1} and λ_{R_2} are also calculated. In this case, the reference wavelengths define a straight line (as in [Figure 6](#)) which is used to calculate A_{R_λ} and $\text{var}(A_{R_\lambda})$ using [Equation 76](#) and [Equation 77](#).

$$A_{R_\lambda} = \frac{1}{\lambda_{R_2} - \lambda_{R_1}} \{ (\lambda_{R_2} - \lambda) A_{R_1} + (\lambda - \lambda_{R_1}) A_{R_2} \} \quad (76)$$

$$\text{var}(A_{R_\lambda}) = \frac{1}{(\lambda_{R_2} - \lambda_{R_1})^2} \left\{ (\lambda_{R_2} - \lambda)^2 \text{var}(A_{R_1}) + (\lambda - \lambda_{R_1})^2 \text{var}(A_{R_2}) \right\} \quad (77)$$

where the terms are the same as for subtraction (see [“Subtract”](#) on page 74).

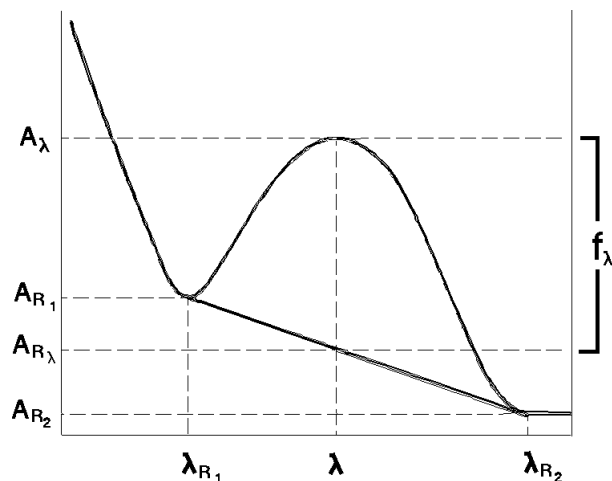


Figure 6 Use of Two Reference Wavelengths

Logarithm (ln)

The logarithm function calculates the natural logarithm of each of the data points (y-values) in the spectrum and places the resulting spectrum in the math result register.

$$y_{\log} = \ln(y) \quad (78)$$

The variances of the logarithmic spectrum, if available, are calculated by [Equation 79](#):

$$\text{var}(y_{\log}) = y^{-2} \cdot \text{var}(y) \quad (79)$$

The threshold parameter is used to avoid large negative y_{\log} values due to very small y-values. Y-values smaller than the threshold are set to the threshold value before the natural logarithm is calculated. The default threshold is 10^{-5} .

All additional information, such as annotations, are preserved unchanged in the logarithmic spectrum.

Reciprocal

The reciprocal function calculates the reciprocal of each of the data points (y-values) in the spectrum and places the resulting spectrum in the math result register.

$$y_{\text{recip}} = \frac{1}{y} \quad (80)$$

The variances of the reciprocal spectrum, if available, are calculated according to [Equation 81](#):

$$\text{var}(y_{\text{recip}}) = y^{-4} \cdot \text{var}(y) \quad (81)$$

The threshold parameter is used to avoid large resulting y_{recip} values due to very small y-values. Y-values smaller than the threshold are set to the threshold value (preserving the sign) before the reciprocal is calculated. Y-values of exactly 0 are set to the reciprocal value of the positive threshold. The default threshold is 10^{-5} .

All additional information, such as annotations, are preserved unchanged in the reciprocal spectrum.

Scalar Add

The scalar add function adds a constant value to each of the data points (y-values) in the spectrum and places the resulting spectrum in the math result register.

$$y_{\text{new}} = y + C \quad (82)$$

The scalar add function can be used to subtract a constant value by using a negative constant.

The variances (if available) and all other additional information, such as annotations, are preserved unchanged in the resulting spectrum.

Scalar Multiply

The scalar multiply function multiplies each of the data points (y-values) in the spectrum by constant value and places the resulting spectrum in the math result register.

$$y_{\text{new}} = y \times C \quad (83)$$

The scalar multiply function can be used to divide by a constant value by using the reciprocal of the desired divisor, $1/C$.

The variances (if available) are multiplied by the square of the constant value:

$$\text{var}(y_{\text{new}}) = \text{var}(y) \times C^2 \quad (84)$$

All other additional information, such as annotations, are preserved unchanged in the resulting spectrum.

Scatter Correction

Solutions that scatter light generally produce a background absorbance spectrum similar to [Figure 7](#), and the spectrum of any absorbing solutes is superimposed on the scatter spectrum.

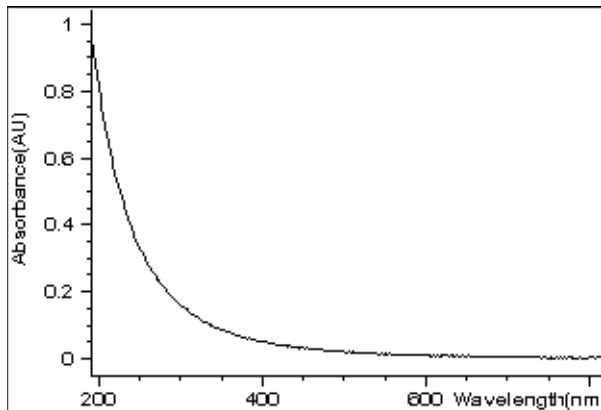


Figure 7 Scatter Spectrum

The Scatter Correction function estimates the contribution due to light scatter and subtracts it from the measured spectrum. The estimation of the scatter spectrum is done by fitting a curve to the data in a wavelength region where the absorbance is due only to scatter. This curve is then extrapolated over the entire spectrum, then subtracted to produce a spectrum of the solutes.

It is assumed that the absorbance spectrum due to scatter obeys [Equation 85](#):

$$A_{\text{scat}} = a \cdot \frac{1}{\lambda^{-b}} = a \cdot \lambda^b \quad (85)$$

Over the wavelength range set in the Scatter Correction Parameter dialog box, the absorbance data is fitted to the linearized form of the equation:

$$\log(A_{\text{scat}}) = \log(a) + b \cdot \log(\lambda) \quad (86)$$

to determine a and b . The scatter spectrum over the whole display range is then calculated using [Equation 85](#), and is subtracted from the measured spectrum to give the scatter-corrected spectrum. Negative absorbance values are removed by performing a scalar addition on the whole scatter spectrum before the coefficients a and b are calculated.

The accuracy of the scatter correction is dependent upon the sizes and shapes of the scattering particles as well as the refractive indices of the particles and sample medium. It also depends upon the selection of a wavelength range in which all absorbance is due to light scattering.

Smooth

The smooth function uses a [“Savitsky-Golay Algorithm”](#) on page 55 to smooth the spectrum and places the resulting spectrum in the math result register. For a description of the derivative function, see [“Derivative”](#) on page 55.

All additional information, such as annotations, are preserved unchanged in the smoothed spectrum.

Spline

The spline function constructs a cubic splined curve through the data points (y-values) in the spectrum and places the resulting spectrum in the math result register. Unlike the smooth function, the spline function does not smooth the spectrum; the splined curve passes through all the original data points, and the spline process inserts additional points between the original ones to produce a continuous curve. The additional data points are calculated by a two-stage process:

Stage 1: Calculating the Second Derivative

The algorithm for calculating the second derivative originates in the following tri-diagonal system of linear equations:

$$\begin{aligned} (x_i - x_{i-1})y''_{i-1} + 2(x_{i+1} - x_{i-1})y''_i + (x_{i+1} - x_i)y''_{i+1} \\ = 6\left(\frac{y''_{i+1} - y''_i}{x_{i+1} - x_i} - \frac{y''_i - y''_{i-1}}{x_i - x_{i-1}}\right) \end{aligned} \quad (87)$$

or, in simplified form,

$$\text{diff1} \cdot y''_{i-1} + 2(\text{diff1} + \text{diff2} \cdot y''_i) + \text{diff2} \cdot y''_{i+1} = 6(\text{quot2} - \text{quot1}) \quad (88)$$

where

diff1 is $x_i - x_{i-1}$

diff2 is $x_{i+1} - x_i$

quot1 is $\frac{y''_i - y''_{i-1}}{\text{diff1}}$

quot2 is $\frac{y''_{i+1} - y''_i}{\text{diff2}}$

The solution is achieved in a two-step process:

- 1 Decomposition and forward substitution.
- 2 Back substitution.

Stage 2: Calculating the Splined Values

The new y-values to be inserted between the original y-values are calculated according to Equation 89:

$$y_x = ((A \cdot \text{diffX} + B)\text{diffX} + C)\text{diffX} + D \quad (89)$$

where

diffX is the distance between the current x-value and the original x-value, $x - x_i$

In Equation 90, the coefficients *A*, *B*, *C*, and *D* are given by:

$$A = \frac{y''_{i+1} - y''_i}{6 \cdot \text{interval}} \quad (90)$$

where

interval is $x_{i+1} - x_i$

i is the current index, starting at 1 and ending at one less than the number of original values

$$B = \frac{y''_i}{2} \quad (91)$$

$$C = \frac{y_{i+1} - D}{\text{interval}} - \frac{1}{6} \cdot \text{interval} \cdot (y''_{i+1} + 4B) \quad (92)$$

where

interval is $x_{i+1} - x_i$

i is the current index, starting at 1 and ending at one less than the number of original values

$$D = y_i \quad (93)$$

The variances of the original spectrum, if available, are deleted.

All additional information, such as annotations, are preserved unchanged in the splined spectrum.

Transmittance

The Transmittance function uses the Exponential, Reciprocal and Scalar Multiply functions to transform absorbance spectra into transmittance spectra using [Equation 94](#). The transmittance spectra are placed in the math result register.

$$T = 100 \cdot 10^{-A} \quad (94)$$

The variances, if available, are transformed by [Equation 95](#):

$$\text{var}(T) = (100 \cdot \ln 10 \cdot 10^{-A})^2 \cdot \text{var}(A) \quad (95)$$

All additional information, such as annotations, are preserved unchanged in the transmittance spectrum.

Peak/Valley Find

The Peak/Valley Find task determines the maxima and minima above a given sensitivity threshold of the data points (y-values) in the defined wavelength range of the spectrum and generates two tables and an annotated spectrum. The tables and spectrum are stored in a temporary register.

The original spectrum is first derivatized (see “[Derivative](#)” on page 55) using the given filter length and a polynomial degree of 3. The derivatized y-values are examined for transitions (change of sign); each transitional y-value is compared with its neighboring values, and if the neighboring values are more extreme, the transitional y-value is compared with the previous transitional y-value. If the difference of both values is greater than or equal to the sensitivity threshold, then the previous transitional point is stored as an extremum; if the difference is less than the sensitivity threshold, then neither point is stored.

If no wavelength range is defined, the task operates over the full range of the selected spectrum.

If no sensitivity threshold is defined, the task operates with a default sensitivity threshold of three times the standard deviation (if available), or zero.

Binary Operations

The binary mathematical functions add, subtract, multiply and divide require two spectra for their operation. If no spectra are selected, or more than two spectra are selected, the operation is aborted and an error message is displayed.

The SumUp function operates on all selected spectra. SumUp requires a minimum of two spectra; if less than two spectra are selected, the operation is aborted and an error message is displayed.

Add

The add function adds two spectra together and places the result in the math result register. The spectrum selected first (spectrum A) is taken as the model and provides the wavelength range (or list) and resolution for the resulting spectrum. Spectrum A is first copied into the math result register, then the y-values (for example absorbance) from the second selected spectrum (spectrum B) are added to the y-values of the spectrum in the math result register as follows:

Spectra of Different Wavelength Resolutions

If the x-values of spectrum A do not completely agree with those of spectrum B

- the y-values of spectrum B are interpolated before adding them to the y-values of the resulting spectrum when the interval of the x-value in spectrum A is less than that of spectrum B.
- the y-values at intermediate x-values in spectrum B are ignored when the interval of the x-value of spectrum A is greater than that of spectrum B, and only the y-values from spectrum B at the x-values corresponding to spectrum A are added to the y-values of the resulting spectrum.

Spectra of Different Wavelength Ranges

If the x-range (wavelength range) of spectrum A is greater than the x-range of spectrum B, the extra y-values in spectrum A remain unchanged in the result.

If the x-range of spectrum A is less than the x-range of spectrum B, the extra y-values in spectrum B are ignored.

If there is no overlap between the x-ranges of the spectra, spectrum A remains unchanged.

Variances

If variances are available in both spectra, the values from the spectrum B are added to those in spectrum A in the same way as for the y-values.

If only spectrum A contains variances, the values remain unchanged in the result.

If only spectrum B has variances, they are ignored.

Additional information, such as annotations, are taken solely from spectrum A; all such items from spectrum B are ignored.

Divide

The divide function divides one spectrum by another and places the result in the math result register. The spectrum selected first (spectrum A) is taken as the model and provides the wavelength range (or list) and resolution for the resulting spectrum. Spectrum A is first copied into the math result register, then the y-values (for example absorbance) of the spectrum in the math result register are divided by the y-values of the second selected spectrum (spectrum B) as follows:

- y-values in spectrum B that are below the threshold are set to the threshold.

Spectra of Different Wavelength Resolutions

If the x-values of spectrum A do not completely agree with those of spectrum B

- the y-values of spectrum B are interpolated before dividing them into the y-values of spectrum A when the interval of the x-value in spectrum A is less than that of spectrum B.
- the y-values at intermediate x-values in spectrum B are ignored when the interval of the x-value of spectrum A is greater than that of spectrum B, and only the y-values from spectrum B at the x-values corresponding to spectrum A are used.

Spectra of Different Wavelength Ranges

If the x-range (wavelength range) of spectrum A is greater than the x-range of spectrum B, the extra y-values in spectrum A remain unchanged in the result.

If the x-range of spectrum A is less than the x-range of spectrum B, the extra y-values in spectrum B are ignored.

Variations

If variations are available in both spectra, the variance of the resulting spectrum is calculated using the [Equation 96](#):

$$\text{var}(y_{\text{result}}) = \frac{1}{2} \cdot \text{var}(y_A) + \frac{y_A^2}{4} \cdot \text{var}(y_B) \quad (96)$$

If only spectrum A contains variations, the values remain unchanged in the result.

If only spectrum B has variations, they are ignored.

Additional information, such as annotations, are taken solely from spectrum A; all such items from spectrum B are ignored.

Multiply

The multiply function multiplies one spectrum by another and places the result in the math result register. The spectrum selected first (spectrum A) is taken as the model and provides the wavelength range (or list) and resolution for the resulting spectrum. Spectrum A is first copied into the math result register, then the y-values (for example absorbance) of the spectrum in the math result register are multiplied by the y-values of the second selected spectrum (spectrum B) as follows:

Spectra of Different Wavelength Resolutions

If the x-values of spectrum A do not completely agree with those of spectrum B

- the y-values of spectrum B are interpolated before multiplying them by the y-values of spectrum A when the interval of the x-value in spectrum A is less than that of spectrum B.
- the y-values at intermediate x-values in spectrum B are ignored when the interval of the x-value of spectrum A is greater than that of spectrum B, and only the y-values from spectrum B at the x-values corresponding to spectrum A are used.

Spectra of Different Wavelength Ranges

If the x-range (wavelength range) of spectrum A is greater than the x-range of spectrum B, the extra y-values in spectrum A remain unchanged in the result.

If the x-range of spectrum A is less than the x-range of spectrum B, the extra y-values in spectrum B are ignored.

Variations

If variations are available in both spectra, the variance of the resulting spectrum is calculated using equation (4.38):

$$\text{var}(y_{\text{result}}) = y_B^2 \cdot \text{var}(y_A) + y_A^2 \cdot \text{var}(y_B) \quad (97)$$

If only spectrum A contains variations, the values remain unchanged in the result.

If only spectrum B has variations, they are ignored.

Additional information, such as annotations, are taken solely from spectrum A; all such items from spectrum B are ignored.

Subtract

The subtract function subtracts one spectrum from another and places the result in the math result register. The spectrum selected first (spectrum A) is taken as the model and provides the wavelength range (or list) and resolution for the resulting spectrum. Spectrum A is first copied into the math result register, then the y-values (for example absorbance) from the second selected spectrum (spectrum B) are subtracted from the y-values of the spectrum in the math result register as follows:

Spectra of Different Wavelength Resolutions

If the x-values of spectrum A do not completely agree with those of spectrum B

- the y-values of spectrum B are interpolated before subtracting them from the y-values of the resulting spectrum when the interval of the x-value in spectrum A is less than that of spectrum B.
- the y-values at intermediate x-values in spectrum B are ignored when the interval of the x-value of spectrum A is greater than that of spectrum B, and only the y-values from spectrum B at the x-values corresponding to spectrum A are subtracted from the y-values of the resulting spectrum.

Spectra of Different Wavelength Ranges

If the x-range (wavelength range) of spectrum A is greater than the x-range of spectrum B, the extra y-values in spectrum A remain unchanged in the result.

If the x-range of spectrum A is less than the x-range of spectrum B, the extra y-values in spectrum B are ignored.

Variances

If variances are available in both spectra, the values from the spectrum B are added to those in spectrum A in the same way as for “Add” on page 70.

If only spectrum A contains variances, the values remain unchanged in the result.

If only spectrum B has variances, they are ignored.

Additional information, such as annotations, are taken solely from spectrum A; all such items from spectrum B are ignored.

SumUp

The sumup function adds several selected spectra together and places the result in the math result register. The spectrum selected first (spectrum A) is taken as the model and provides the wavelength range (or list) and resolution for the resulting spectrum. Spectrum A is first copied into the math result register, then the y-values (for example absorbance) from the subsequently-selected spectra are added to the y-values of the spectrum in the math result register as follows:

Spectra of Different Wavelength Resolutions

If the x-values of spectrum A do not completely agree with those of the other spectra:

- the y-values of the other spectra are interpolated before adding them to the y-values of the resulting spectrum when the interval of the x-value in spectrum A is less than that of the other spectra.
- the y-values at intermediate x-values in the other spectra are ignored when the interval of the x-value of spectrum A is greater than that of the other spectra, and only the y-values from the other spectra at the x-values corresponding to spectrum A are added to the y-values of the resulting spectrum.

Spectra of Different Wavelength Ranges

If the x-range of any of the other spectra in the sum are less than that of spectrum A the y-value contributions from non-existent x-values are zero.

If the x-range of spectrum A is less than the x-range of all other spectra in the sum, the extra y-values in the other spectra are ignored.

Variances

If spectrum A contains variiances, the variance values from those other spectra in the sum that contain variiances are added to those of spectrum A; the contribution of any spectra that do not contain variiances is zero.

If spectrum A does not contain variiances, the variance values of all other spectra in the sum are ignored.

Additional information, such as annotations, are taken solely from spectrum A; all such items from the other spectra in the sum are ignored.

Spectral Match

The spectral match function compares two spectra by linear regression. The match factor is calculated over the common wavelength range of the two spectra.

The match factor is calculated by Equation :

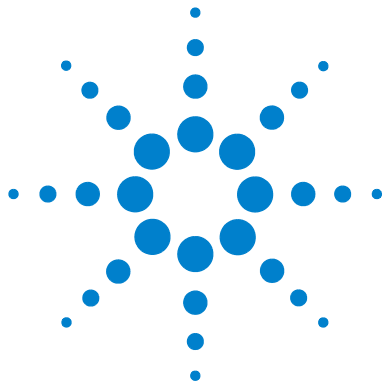
$$\text{Match Factor} = \frac{10^3 \left(\sum y_A \cdot y_B - \left(\frac{\sum y_A \sum y_B}{n} \right) \right)^2}{\left(\sum y_A^2 - \frac{(\sum y_A)^2}{n} \right) \left(\sum y_B^2 - \frac{(\sum y_B)^2}{n} \right)} \quad \text{where}$$

y_A , y_B are the weighted intensity values of spectrum A and spectrum B respectively. The weighted intensity values, w , are calculated by multiplying them by the reciprocal of the square roots of the

$$\text{variances: } w = \frac{y}{\sqrt{\text{var}}}$$

n is the number of data points

At the extremes, a match factor of 0 indicates totally dissimilar spectra and a match factor of 1000 indicates identical spectra. Generally, values above 990 indicate an acceptable degree of similarity; values between 900 and 990 indicate some similarity, but with a degree of doubt; values below 900 show that the spectra are different.



5 Optimization

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Compare (Normalization)

The Compare by Normalization task compares two spectra by matching amplitudes exactly at two points, the normalization point and the internal reference point, then subtracting the second spectrum from the first. The subtraction process is identical with the Subtract function (see “[Subtract](#)” on page 74). The result is a residual spectrum and a table containing the mathematical results of the normalization. The residual spectrum is displayed over the common wavelength range of the two spectra; the fit value is calculated over the wavelength range specified in the Compare (Normalization) Parameters dialog box (by default, the common wavelength range of the two spectra).

The spectra are normalized using a normalization factor, N , which is the ratio of the data values (y -values) of the two spectra at the normalization points, y_{np} corrected by the data values (y -values) at the internal reference points, y_{irp} :

$$N = \frac{(y_{np_B} - y_{irp_B})}{(y_{np_A} - y_{irp_A})} \quad (98)$$

where

y_{irp} is the measured data value at the internal reference point

y_{np} is the measured data value at the normalization point

A, B are the respective spectra A and B

The normalization factor is then used to calculate the y -values for the comparison result plot:

$$y_{i, \text{result}} = \frac{y_{i, B} - (N \cdot y_{i, A})}{y_{np_B}} \quad (99)$$

where

y_i is the data value of the i^{th} data point

y_{np} is the measured data value at the normalization point

Division of the data values by the data value of spectrum B at the normalization wavelength effectively sets the value of both spectra to unity at the normalization wavelength. The difference is then independent of the amplitudes of the original spectra.

The fit value, which is given in the results table, is calculated as the sum of the squares of the result divided by the number of data points minus one (see [Equation 100](#)) if no variances are available or by using the variances of all measured data points as shown in [Equation 101](#).

For spectra without variance data the fit value is calculated:

$$F = \frac{\sum_{i=1}^n y_{i, \text{result}}^2}{n - 1} \quad (100)$$

where

n is the number of data points

and for spectra with variances:

$$F = \frac{\sum_{i=1}^n y_{i, \text{result}}^2}{n \cdot \left(\frac{V_{irp_A}}{2} + \frac{V_{irp_B}}{2} \right) + \sum_{i=1}^n \left(\frac{V_{i,A}}{2} + \frac{V_{i,B}}{2} \right)} \quad (101)$$

where

V_{irp} is the measured variance at the internal reference point

V_{np} is the corrected data value at the normalization point

V_i is the measured variance of a data value

5 Optimization

Compare (Normalization)

Two identical spectra give a fit value of 0; the higher the fit value, the less similar the spectra.

Two spectra must be selected before the compare by normalization task is executed. If the number of spectra selected is more or less than two, execution is aborted and an error message is displayed.

A normalization wavelength within the common wavelength range of the two spectra must be specified. If the normalization wavelength is outside the common wavelength range of the spectra, execution is aborted and an error message is displayed. It is not necessary for the normalization wavelength to be within the wavelength range specified for the calculation of the fit value.

The internal reference wavelength is optional, but if specified must be within the wavelength range of one of the spectra. If the internal reference wavelength is outside the wavelength range of both spectra, execution is aborted and an error message is displayed. It is not necessary for the internal reference wavelength to be within the wavelength range specified for the calculation of the fit value.

Compare (Regression)

The compare by linear regression task compares two spectra by linear regression of the first spectrum (spectrum A) onto the second (spectrum B). The parameters allow spectrum B to be shifted in wavelength before the comparison is made. A negative value shifts spectrum B to a lower wavelength, a positive value shifts it to a higher wavelength. The result of the comparison is a residual spectrum, a table containing the mathematical results of the comparison and a plot of the amplitude values of each spectrum at each wavelength. The residual spectrum and the amplitude plot are displayed over the common wavelength range of the two spectra; the match factor is calculated over the wavelength range specified in the Compare (Regression) Parameters dialog box (by default, the common wavelength range of the two spectra).

The y-values of spectrum B are interpolated into those of spectrum A. This gives sets of values: pairs if neither spectrum contains variances, triplets if only one spectrum contains variances or quadruplets if both spectra contain variances. If one or more of the values in a set is invalid, or both absorbance values lie below the specified threshold, this set is ignored in the comparison. If variances are not available, a least squares fit is calculated; if variances are available for both spectra, a weighted least squares fit (maximum likelihood) is calculated. The slope and intercept of the fitted line are reported in the Compare Results (Regression) table.

5 Optimization

Compare (Regression)

The match factor, also reported in the Compare Results (Regression) table is calculated by [Equation 102](#):

$$\text{Match Factor} = 1000 \cdot \frac{\left(\sum_{i=1}^n y_{i,A} \cdot y_{i,B} - \frac{\left(\sum_{i=1}^n y_{i,A} \right) \left(\sum_{i=1}^n y_{i,B} \right)}{n} \right)^2}{\left(\sum_{i=1}^n y_{i,A}^2 - \frac{\left(\sum_{i=1}^n y_{i,A} \right)^2}{n} \right) \left(\sum_{i=1}^n y_{i,B}^2 - \frac{\left(\sum_{i=1}^n y_{i,B} \right)^2}{n} \right)} \quad (102)$$

where

y_A, y_B are the data values of spectrum A and spectrum B respectively.

If variances are available, the intensity values, y , in [Equation 102](#) on page 82 are replaced by weighted intensity values, w , calculated by multiplying them

by the reciprocal of the square roots of the variances: $w = \frac{y}{\sqrt{\text{var}}}$

n is the number of data points

At the extremes, a match factor of 0 indicates totally dissimilar spectra and a match factor of 1000 indicates identical spectra. Generally, values above 990 indicate an acceptable degree of similarity; values between 900 and 990 indicate some similarity, but with a degree of doubt; values below 900 show that the spectra are different.

Compose

The compose task calculates a simulated spectrum from specified amounts of the selected spectra. The synthesized spectrum is appended to the Samples register. The selected spectra are inserted into a Concentration Matrix table. If no analyte information is available for any of the selected spectra, the table consists of the sample name and a scaling factor (default value of 1). The relative amounts of the selected spectra in the synthesized spectrum are defined by the factor values. If analyte information is available for one or more of the selected spectra, the table includes fields for the values of all analytes in each sample. The relative amounts of the analytes from the selected spectra in the synthesized spectrum are defined by the entries in the Val.ToUse fields.

The compose task operates by performing a scalar multiplication of each spectrum by its scaling factor (see “[Scalar Multiply](#)” on page 64), then a SumUp of the scaled spectra (see “[SumUp](#)” on page 75).

Variances (if available) are treated identically to the treatment of variances in the relevant mathematical operations.

Evaluate Standards

The Evaluate Standards task carries out a single component analysis calibration on a set of standards (see [Chapter 2](#), “Single Component Analysis (SCA)”), for an explanation of the calculations and the equations used), applying the specified calibration parameters to all specified wavelengths or wavelength ranges. The task calculates the correlation coefficient at each wavelength using [Equation 22](#) on page 30 (for the least squares method) or [Equation 24](#) on page 31 (for the maximum likelihood method), and the uncertainty at each wavelength using [Equation 30](#) on page 32. The results are plotted against wavelength, and give a visual indication of the quality of the calibration. The best calibration is achieved over the wavelength range where the uncertainty is at a minimum, or the correlation coefficient is nearest to 1.

Optimize Wavelengths

The Optimize Wavelengths task carries out a single component analysis on a sample (see [Chapter 2](#), “Single Component Analysis (SCA)”), for an explanation of the calculations and the equations used), applying the specified calibration parameters to all specified wavelengths or wavelength ranges. The task calculates the analytical result at each wavelength using the calculated calibration coefficients and displays a plot of analytical result against wavelength. The optimum wavelength is either where the analytical result is nearest to the correct value (if the correct value is known, for example in a standard) or (if the correct value is not known) the wavelength giving the lowest concentration. This analysis of the Optimize Wavelengths result makes the assumption that any interference will cause an increase in the analytical result.

Compare Calibrations

The Compare Calibrations task displays the results of two different calibrations of the same set of standards so that they can be directly compared. The calibrations are set up using the extended analysis facilities (either multiple analyses or a confirmation analysis), and can be either single component analysis or multicomponent analysis. The task displays selected parameters and the results that have been calculated as described in [Chapter 2](#), “Single Component Analysis (SCA)” and [Chapter 3](#), “Multicomponent Analysis (MCA)”.

Test Method

The test method task is used to produce a statistical analysis of multiple sets of analysis results. The task calculates a mean value and a standard deviation of all available analysis results; for multiple component analyses with multiple analytes, each analyte is calculated and displayed separately, for multiple data analyses, each data analysis is also calculated and displayed separately.

The test method results for each analyte and each data analysis are displayed individually as a plot of value against sample number, and summarized in tabular form showing the mean value and standard deviation. The mean value is denoted on the individual plots by a horizontal line at the mean value. The tabular display shows all results for all analyses.

Test method can be used to measure the precision of a method, by calculating the standard deviation of the results of a sample that has been measured multiple times. The task can also be used to compare the results from different data analysis methods.

The equation used to calculate the mean in the Test Method task is:

$$\bar{R} = \frac{R_1 + R_2 + R_3 + \dots + R_n}{n} \quad (103)$$

where

\bar{R} is the mean value of all results of the n analyses

$R_1 \dots R_n$ are the individual results of the n analyses

5 Optimization Test Method

The mean from [Equation 103](#) is then used in the calculation of the standard deviation:

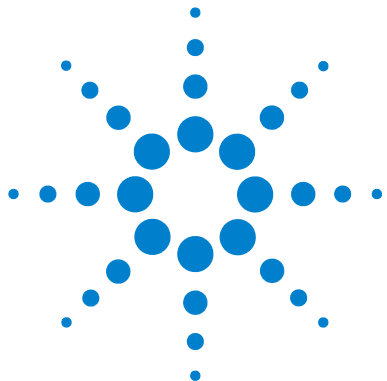
$$s = \sqrt{\frac{\sum_{i=1}^n (R_i - \bar{R})^2}{(n-1)}} \quad (104)$$

where

s is the standard deviation of the results of the n analyses

R_i are the individual results of the n analyses

\bar{R} is the mean value of all results of the n analyses



6 Automation

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Overview

The automatic analysis of a series of samples runs under the control of two memory-resident files:

- the automation table, which contains a program defining the order of the procedures that will take place during the analysis,
- the sample table, which contains information about the samples to be run during the analysis.

The automation table and sample table are interconnected so that, for example, when a sample is identified in the automation table, its information is gathered automatically from the sample table.

When the advanced software is started, the default automation table and sample table are loaded into memory; both tables are empty. Completed automation tables and sample tables can be saved to disk and recalled for reuse. Both tables are stored in the subdirectory AUTOMAT.

The Automation Table

The current automation table is held in a register called Automation. The Automation register contains the following information about the automation table:

- The **Title** is the name of the automation file. This is the name under which the automation table was stored and recalled; the default automation table has a null name.
- The **Source Id** identifies the source of the sample (for example the autosampler vial number). This source is sampled when this line is run. If the Source Id is zero, the measure action is carried out without activation of the sampling system or prompt to the user.
- The **Sample Name** identifies the sample in the sample table (see “[The Sample Table](#)” on page 93). The Sample Name is copied to the Raw Data register along with the measured spectrum.
- **Action** identifies the action that is taken when this line is run. [Table 5](#) shows the actions that are available for automation.
- **Parameter** provides any additional information that is necessary for the Action to be executed. Refer to [Table 6](#) for details of those Actions that demand an entry in the Parameter field.
- **From Line** identifies the number of the line in the automation table that starts the automation process. The start line is by default 1.
- **To Line** identifies the number of the line in the automation table that ends the automation process. The end line is generally the last line of the automation table.
- The **Auto Desc** item contains the contents of the Edit Automation Information dialog box. This is a free-style description of the automation process.

Table 5 Actions Available for Automation

Command	Action
Measure Blank	Measures the blank spectrum defined by the source.
Measure Sample	Measures the sample defined by the sample name and source.
Measure Standard	Measures the standard defined by the sample name and source.
Measure Control	Measures the sample defined by the sample name and source as a control. The acceptance limit (%) is specified in Parameter.
Measure Auxiliary	Measures the sample defined by the sample name and source into the auxiliary register.
Calibrate	Performs a calibration using the current method's calibration parameters.
Analyze	Performs a data analysis using the current method.
Method Report	Produces a method report using the current method report parameters.
Result Report	Produces a result report using the current result report parameters.
Calibration Report	Produces a calibration report using the current calibration report parameters.
Clear Samples	Clears the samples register.
Clear Standards	Clears the standards register.
Clear Auxiliary	Clears the auxiliary register.
Load Method*	Loads the method specified in Parameter.
Load Samples*	Loads data specified in Parameter into the samples register.
Save Samples*	Saves the data in the samples register to the file specified in Parameter.
Load Standards*	Loads data specified in Parameter into the standards register.
Save Standards*	Saves the data in the standards register to the file specified in Parameter.
Load Auxiliary*	Loads data specified in Parameter into the auxiliary register.
Save Auxiliary*	Saves the data in the auxiliary register to the file specified in Parameter.

Table 5 Actions Available for Automation (continued)

Command	Action
Save Results to ECM ^{*,**}	Save results to the Enterprise Content Management (ECM) system
Load Method from ECM ^{*,**}	Load method from the Enterprise Content Management (ECM) system
Set Method Path [*]	Sets the path for loading and saving methods to that specified in Parameter.
Set Data Path [*]	Sets the path for loading and saving data files to that specified in Parameter.
Set Temperature [*]	Sets the temperature specified in Parameter on the optional Agilent 89090A Peltier temperature control accessory
User Macro [*]	Starts the macro specified in Parameter.

* Requires an entry in the Parameter field.

** Available only if ECM software module installed

Table 6 Automation Actions Requiring a Parameter

Action	Parameter Required
Load Method	Requires the name of the method to load. The method must be in the default path (or a path previously specified using Set Method Path).
Load Samples	Requires the name of the file to load into the Samples register. The file must be in the default path (or a path previously specified using Set Data Path).
Save Samples	Requires the name of the file to which to save the sample data. The file must be in the default path (or a path previously specified using Set Data Path).
Load Standards	Requires the name of the file to load into the Standards register. The file must be in the default path (or a path previously specified using Set Data Path).
Save Standards	Requires the name of the file to which to save the Standards. The file must be in the default path (or a path previously specified using Set Data Path).

Table 6 Automation Actions Requiring a Parameter (continued)

Action	Parameter Required
Load Auxiliary	Requires the name of the file to load into the Auxiliary register. The file must be in the default path (or a path previously specified using Set Data Path).
Save Auxiliary	Requires the name of the file to which to save the sample data. The file must be in the default path (or a path previously specified using Set Data Path).
Save Results to ECM	Save results to the Enterprise Content Management (ECM) system
Load Method from ECM	Load method from the Enterprise Content Management (ECM) system
Set Method Path	Requires the path from which methods will be loaded.
Set Data Path	Requires the path from which data will be loaded and to which data will be saved.
Set Temperature	Requires the temperature to set on the Agilent 89090A Peltier temperature control accessory.
User Macro	Requires the name of the macro to run. The macro must already be loaded in memory

The Sample Table

The current sample table is held in a register called SampleLog. The SampleLog register contains the following information about the sample table:

- The **Title** is the name of the sample table file. This is the name under which the sample table was stored and recalled; the default sample table has a null name.
- The **Sample Name** identifies the sample, standard or control. Each sample occupies a separate line in the sample table, and has an associated analytes table. The Sample Name is the cross-reference for the sample identity in the automation table (see “[The Automation Table](#)” on page 89) and the information from the sample table is used in any calculations defined in the automation table.
- The **Solvent Name** optionally contains the name of the solvent. The Solvent Name is copied to the Raw Data register along with the measured spectrum.
- The **Comment** item optionally contains a free-style comment about the sample. The Comment is copied to the Raw Data register along with the measured spectrum.
- The **Analytes Table** is the core of the sample table; it contains all the information about the analytes in the sample. Each row of the analytes table contains information about a separate analyte. The table contains four columns:

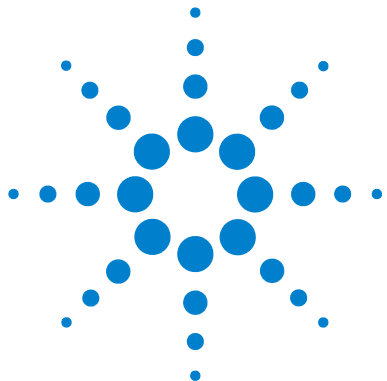
Analyte Name identifies the analyte.

Value gives the concentration of the analyte in the sample.

Std Dev optionally provides information about the errors in the value from the preparation method.

Unit gives the units of the value; this is carried through into the calculation of the analytical results.

6 Automation
The Sample Table



7 Reports

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Overview

Advanced Mode allows three predefined reports to be generated.

- The Results Report is a report of the current evaluation results; it optionally includes a method report.
- The Calibration Report is a report of the current calibration results.
- The Method Report is a report of the parameters of the current method; it optionally includes a calibration report.

In addition to the predefined reports, the customized report layout task allows report templates to be set up, which can be used to print customized reports.

The predefined reports can be output directly to a printer, to the screen or printed as a text file to a file with the extension .TXT. Reports that are printed to files are stored in the REPORTS subdirectory of the instrument's subdirectory.

In addition to the three formal reports and the customized reports, the software allows any selected window to be printed, and any view (all windows) to be printed. The windows and views are formatted as reports.

The appearance of the report is affected by two sets of parameters:

- The report configuration parameters control the margins, and indents for textual data. The annotation limit, which is the maximum number of spectra that are annotated, is also controlled by the report configuration parameters. A one-line header text (for example, a company name, can also be specified in the report configuration.

NOTE

Make sure the header line entered does not exceed the number of characters/line for your current printer. Additional characters are automatically truncated.

If a spectrum plot contains less spectra than the annotation limit, all spectra are annotated. If the spectrum plot contains more spectra than the annotation limit, none of the spectra are annotated.

- The printer setup parameters control the target printer and the paper size and orientation.

Results Report

The contents of the results report are determined by the selected check boxes in the Results Report Parameter dialog box. The information sources of the results report contents are given in [Table 7](#).

Table 7 Sources of Information for the Results Report

Check Box Title	Report Section Title	Information Sources
	Results Report header	Name and path of method file Number of samples Name of operator
Sample Information	Sample number	Contents of the relevant line in the Sample Spectra table
Sample Spectra	Sample Spectrum	The spectrum of the sample, taken from the Sample Spectra register
Processed Sample Spectra	Processed Sample Spectrum	The sample spectrum after spectral processing, taken from the Processed Sample Spectra register
Used Wavelength Results	Used Wavelength Results	The function result from the Wavelength Results register
Evaluation Results	Sample number	The value of the analysis result from the Evaluation Results register
Statistical Information	Sample number	Statistical information specific to multicomponent analysis
Residual Spectra	Residual Spectrum	The residual spectrum of each of the samples of a multicomponent analysis
Confirmation Results	Sample number	Results of data analysis and confirmation analyses
Include Method Report	Method Report Parameter	A method report containing the selected items (see Table 9 for contents)
All Sample Spectra Overlaid	Sample Spectra Overlaid	The contents of the Sample Spectra register
Summary	Results Summary	The Evaluation Results table for single analysis The All Analysis Results table for multiple analyses The Confirmation Analysis Results table for confirmation analysis
Evaluation Result Statistics	Method Summary	Mean value, Minimum value, Maximum value, Standard Deviation and % Relative Standard Deviation of the sample results

Calibration Report

The contents of the calibration report are determined by the selected check boxes in the Calibration Report Parameter dialog box. The information sources of the calibration report contents are given in [Table 8](#).

Table 8 Sources of Information for the Calibration Report

Check Box Title	Report Section Title	Information Sources
	Calibration Report header	Number of standards
Standard Spectra	Standard Spectrum	The spectrum of the standard, taken from the Standard Spectra register
Processed Standard Spectra	Processed Standard Spectrum	The spectrum of the standard after spectral processing, taken from the Processed Standard Spectra register
Path Length Table		Values extracted from the contents of the Standard Spectra table
Data Analysis Parameters	Data Analysis	Spectral processing steps, parameters for each step from the Parameter dialog box Use Wavelength(s) parameters from the dialog box of the selected wavelength specification dialog box (Single, List, Range, Analytical Function) Evaluation type, parameters from SCA or MCA Parameter dialog box
Coefficients	Coefficients	SCA: the values of the coefficients in the calibration curve equation MCA: a table of the coefficients for each component at each wavelength
Used Wavelength Results	Used Wavelength Results	The function results from the Wavelength Results register

Table 8 Sources of Information for the Calibration Report (continued)

Check Box Title	Report Section Title	Information Sources
Calibration Table(s) of Analytes	Calibration Table of (Analyte Name)	SCA: Values extracted from the SCA Calibration Results table MCA: Values extracted from the Analytes of Standards tables
Curve	Calibration Curve (Data Analysis) [†]	A hard copy of the Calibration Curve window
Residual Spectra	Residual Spectra [‡]	A hard copy of the Residuals of Processed Standards window
Diagnostics	SCA: SCA Summary	The contents of the SCA Summary table
	MCA: Pure Analyte Spectra	A hard copy of the MCA Pure Component Spectra window

* For multiple analyses and confirmation analysis, each set of data analysis parameters is reported under a separate header.

† There is no calibration curve output for multicomponent analysis

‡ There is no residual spectra output for single component analysis

Method Report

The contents of the method report are determined by the selected check boxes in the Method Report Parameter dialog box. The information sources of the method report contents are given in [Table 9](#).

Table 9 Sources of Information for the Method Report

Check Box Title	Report Section Title	Information Sources
	Method Report header	Name and path of method file Date of last update of method Name of operator who made the last update Software product and revision numbers
Information	Method Information	Contents of the Edit Method Information dialog box
Checklist	Method Checklist	Contents of the Method Checklist: Name of the pre measure macro Name of the post measure macro Name of the file in which the spectra are automatically stored Type of analyses (Single, Multiple, Confirmation) and number
Instruments/Acquisition	Sampling System	Configured sampling system and sampling system parameters from the sampling system's Parameter dialog box
	Spectrophotometer	Spectrophotometer type and acquisition parameters from Spectrophotometer Parameter dialog box
	Temp. Controller (optional)	Parameters from Agilent 89090A Parameter dialog box
Data Analysis*	Data Analysis	Spectral processing steps, parameters for each step from the Parameter dialog box Use Wavelength(s) parameters from the dialog box of the selected wavelength specification dialog box (Single, List, Range, Analytical Function) Evaluation parameters (if any) from the Equation Parameter dialog box

Table 9 Sources of Information for the Method Report (continued)

Check Box Title	Report Section Title	Information Sources
Report	Method Report Parameter	State of the check boxes in the Method Report Parameter dialog box
	Result Report Parameter	State of the check boxes in the Results Report Parameter dialog box
Include Calibration Report	Calibration Report Parameter	A calibration report containing the selected items (see Table 8)

* For multiple analyses and confirmation analysis, each set of data analysis parameters is reported under a separate header.

7 Reports
Method Report

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In This Book

This manual describes the data processing operations of the advanced software for the Agilent ChemStation for UV-visible spectroscopy. It describes the processes and transformations that the spectrophotometric data undergo between the time that they are acquired in the spectrophotometer and their output on the Agilent ChemStation display or printer. The manual is designed to enable you to follow good laboratory practice (GLP) guidelines. Using the information in the manual, you will be able to understand the data processing calculations from beginning to end and perform the data evaluations manually.

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