

SMARTFORMULA MANUALLY COMMAND

The **SmartFormula Manually** command is used to calculate possible formulae for a given m/z value. The calculated molecular formulae are listed in the Manual SmartFormula List in the **SmartFormula Manually** dialog and also in the SmartFormula List shown in the **SmartFormula** tab of the Spectrum Data window. The current SmartFormula Manually result can be viewed in a 'Mass Spectrum SmartFormula Report' and will be saved to the respective mass spectrum when the analysis is saved. The theoretical isotopic pattern for a selected formula can be simulated and saved to the original spectrum, too.

The calculation of molecular formulae is based on the parameters settings defined in the **SmartFormula Manually** dialog and the isotope masses, their abundances and the valencies defined in the **SmartFormula** dialog. The valencies used for calculating the rings and double bonds, the electron configuration and the nitrogen rule you can quickly inspect in the **SmartFormula** dialog, too.

The command opens the **SmartFormula Manually** dialog to enter the m/z value for which possible formulae should be calculated and to specify how calculation should be performed.

SmartFormula Manually dialog (here with the minimum and maximum atom numbers used in the example below already entered)

Lower formula and Upper formula

Enter all elements with their allowed minimum (**Lower formula**) and maximum (**Upper formula**) atom numbers on which formula calculation should be based. Formula calculation will start at the formula entered in **Lower formula** and stop at the formula entered in **Upper formula**.

Note: For m/z values < 2000, the elements C, H, N and O are considered implicitly.

Tip: The PeriodicTableEditor application allows defining new isotopically labeled elements. This application is available via Windows **Start** menu > **All Programs** > **Bruker Daltonics** > **Utilities** > **PeriodicTableEditor**.

Hints on how to enter minimum and maximum formulae:

- If you want to generate formulae for m/z values < 2000 and if the element C, H, N or O should be excluded from calculation, you must explicitly set the respective element to '0'. For example, if there should be no N in the formulae you have to enter 'N0'.
- Sorting of elements is not needed.
- Hard brackets can be used to enter fixed atom ratios that should not be resolved. For example, when entering '[N3P3O6]CHF' for **Lower formula** and '[N3P3O6]C100H21F80' for **Upper formula**, then calculation will use exactly 3 N, 3 P and 6 O but 1 to 100 C, 1 to 21 H and 1 to 80 F.
- Chemical notation can be used. For example, '(CH3)6' will be resolved to C6H18.

(Allowed elements and min/max atom numbers)

Lists the constraints for generating formulae as specified in **Lower formula** and **Upper formula**, i.e. the elements and their allowed

min/max atom numbers. Within these bounds molecular formula calculation will occur.

Adducts, pos.

Select adducts for mass spectra of positive polarity to take into consideration simultaneously.

Adducts, neg.

Select adducts for mass spectra of negative polarity to take into consideration simultaneously.

Collect adducts


Check this option if for each finding with the assumed adduct all other checked adducts should be tested on the left and the right hand side in the spectrum and all results of the same neutral formula but different adducts should be scored together.

Measured m/z

Enter the measured m/z value for which possible formulae should be calculated. You can enter the value as follows:

- Type the m/z value into the box.

or

- Select the m/z value in the selected mass spectrum. For this, position the SmartFormula Manually cursor  at the desired data point and click the left mouse button. This enters the corresponding m/z value: **Note:** If **Generate immediately** is set, this immediately starts generating formulae.

Tolerance

Define an absolute or relative tolerance for the specified m/z value, which determines the total mass range within which the m/z of the calculated formulae must lie. Enter the desired value and select the corresponding unit (**mDa** or **ppm**) from the drop-down list on the right.

Charge

For multiple charged ions, enter the assumed charge the ion of the **Measured m/z** has.

Please consider the sign of the charge. The mass of the electron is not negligible for high mass precision instruments. Switching between +1 and -1 makes a difference of two electrons or 1.1 mDa which means 2 ppm for a molecule with 548 Da.

Automatically locate monoisotopic peak

Check this option if the monoisotopic peak that corresponds to the **Measured m/z** should be located automatically. The algorithm will search to the left and right of the selected **Measured m/z** for appropriate sum formulae with their corresponding monoisotopic peaks.

To use this option select the largest peak of the pattern. If search was successful, formulae that correspond to the found monoisotopic peak (s) will be entered in the result list, too. For formulae with a monoisotopic peak different from the selected peak the monoisotopic mass differs by a few mDa from the selected mass. The mass error nevertheless is calculated for the selected peak and the exact theoretical mass of that peak based on the formula from Alan L. Rockwood et al. (J.Am.Soc. Mass Spectrom. 2004, 15, 12-21).

Maximum number of formulae

Enter the maximum number of formulae to be calculated at most. The formulae with the smallest relative error based on the measured m/z will be picked out.

Check rings plus double bonds

Check this option if possible formulae should be checked whether the number of rings and double bonds present keeps within the bounds specified in **Minimum** and **Maximum**. This will exclude all possible formulae with deviating double bond equivalence numbers.

The rings plus double bonds number is calculated from the number of atoms and their valency:

$$R + db = \frac{2 + \sum n_{atom} \times (valency_{atom} - 2)}{2}$$

The valency of C and Si is 4, for N and P a valency of 3 is assumed. For O and S it is 2, for H and the halogen atoms it is 1. For this limited set of atoms the equation simplifies to

$$R + db = n_{(C)} - \frac{1}{2} n_{(H,D,F,Cl,Br,I)} + \frac{1}{2} n_{(N,P)} + 1$$

All other elements are considered too. Double bonds occurring with higher valence states like 4 or 6 for sulfur cannot be taken into account since these valence states are not known.

Minimum

Enter the minimum number of rings plus double bonds to be contained in a formula at least. This number may be negative for protonated clusters.

Maximum

Enter the maximum number of rings plus double bonds to be contained in the formula at most.

Electron configuration

Select the kind of electron configuration to be applied:

even. Generates only molecular formulae having an even electron configuration.

odd. Generates only molecular formulae having an odd electron configuration.

both. Generates molecular formulae of both electron configurations.

The electron configuration is calculated under the assumption that we have molecular ions. The electron configuration is even, if $[n(\text{atom}) \times \text{valency}(\text{atom}) + z(\text{ion})]$ is even.

Filter H/C element ratio

Check this option if possible molecular formulae should be filtered for their ratio of the elements H and C. Only formulae that comply with the H/C ratios specified in **Minimum H/C ratio** and **Maximum H/C ratio** will be displayed.

Minimum H/C ratio

For the H/C ratio filter enabled, enter the minimum H/C ratio to be applied.

Maximum H/C ratio


For the H/C ratio filter enabled, enter the maximum H/C ratio to be applied.

Estimate carbon number

Check this option if the minimum number of carbon atoms to be used in generating molecular formulae should be estimated by the program. The estimated number will be entered in **Lower formula** after generating formulae has been started. If **Lower formula** already contains a manually entered carbon number, the manually entered and the estimated number will be compared and the higher of both carbon numbers will be taken over. This option should be set if the monoisotopic peak could not be determined reliably e.g. in case of a large number of carbon atoms or metal organic compounds.

For estimating the carbon number, the following assumptions are made: The selected m/z value corresponds to the m/z of the monoisotopic peak. The next peak that corresponds to the isotopomer with one ^{12}C less and exactly containing one ^{13}C , is ± 1.003355 apart from the selected m/z value. From the intensity ratio of both peaks, a carbon number is estimated and rounded down. This number is then used as the minimum carbon number in generating formulae.

Generate immediately

Check this option if generating SmartFormula should immediately start when clicking with the SmartFormula Manually cursor  on an m/z value in the selected mass spectrum.

Generate

Generates for the mass range specified by the **Measured m/z** and **Tolerance** values possible molecular formulae that meet the specified parameter settings and enters them with corresponding data in the molecular formula list. If **Show Pattern** is already turned on, the pattern of the first formula in this list will be displayed below the original spectrum and entered in the Analysis List as a subentry of the original spectrum.

(Manual SmartFormula list)

Lists the generated formulae for the given **Measured m/z** value including various score, error and statistical values. The layout of the Manual SmartFormula List is based on the current settings in the **Layouts** - tab of the **Method Parameters** dialog. You can edit the layout of the list to show e.g. additional statistical values and rankings. For this, right-click in the list, select from the context menu and change the SmartFormula List layout settings as desired. Changing the layout settings will update the list immediately.

The following data is displayed in the example below :

Column	Description
Meas. m/z	Measured m/z value.
#	Running number of formula (for each m/z).
Formula	Sum formula.
Score	Score of the formula (a value between 0 and 100%).
m/z	Calculated m/z of the formula.
err [mDa]	Deviation between measured mass and theoretical mass of the selected peak in [mDa].
err [ppm]	Deviation between measured mass and theoretical mass of the selected peak in [ppm].
mSigma	Sigma is a rate for the agreement of the theoretical and measured isotopic pattern of the mass peak of interest. It combines the standard deviation of the masses and intensities for all isotopic peaks. The values are given in [milliSigma] and lower numbers indicate a better fit.
rdB	Number of rings and double bonds in the formula.
e Conf	Indicates whether the electron configuration is even or odd.
N-Rule	Indicates whether the nitrogen rule is fulfilled.
Adduct	Considered adduct ion.

Copy to SmartFormula Parameters

Copies the current parameter settings for SmartFormula Manually to the **Chemistry** - tab of the **Method Parameters** dialog. This allows saving these parameter settings in a method.

Show Pattern

Allows showing and removing again the pattern of a formula selected in the molecular formula list.

On first clicking this button, the show pattern function is turned on which results in the pattern of the currently selected formula being displayed below the original spectrum and entered in the Analysis List as a subentry of the original spectrum. When selecting another formula from the list, the previous pattern is replaced by the current one; the same occurs to the Analysis List entry. If on performing formula generation this function is already turned on, the pattern of the first formula in the list will be displayed and entered automatically.

On clicking the button again, the show pattern function is turned off which results in removing the currently shown pattern and its Analysis List entry.

If you want to preserve one of the patterns to save it to the original spectrum, select the respective formula and close the **SmartFormula Manually** dialog without turning off the show pattern function. If you want to preserve more than one pattern, repeat generating formulae operation using the same parameter settings, show the desired additional pattern and close the dialog without turning show the pattern function off.

SmartFormula Manually

Lower formula: $C_{12}H_4F_4N_3O_6P_3$

Upper formula: $C_{20}H_{13}P_3O_6F_{10}$

C 12-60, F 1-100, H 1-19, N 3-n, O 6-6, P 3-3

Note: for m < 2000 the elements C, H, N, and O are considered implicitly.

Adducts, pos: M+H, M+H4, M+Na, M+K

Adducts, neg: M-H, M+Na+H2

Measured m/z: 0 Tolerance: 4 mDa Charge: 1

Meas. m/z	#	Ion Formula	m/z	err [mDa]	err [ppm]	mSigma	# mSigma	rb	N-Rule	e
922.0095	1	C14H17F218NaO6P3	922.0072	-2.3	-2.5	3.9	1	1.5	ok	ev
	2	C12H14F18N15NaO6P3	922.0069	-2.6	-2.8	3.9	2	5.5	ok	ev
	1	C12H10F18N12IO6P3	922.0091	-0.4	-0.4	8.7	1	12.5	ok	ev
	2	C14H13F18N15O6P3	922.0093	-0.2	-0.2	8.7	2	8.5	ok	ev
	1	C12H10F18N12IO6P3	922.0091	-0.4	-0.4	8.7	1	12.5	ok	ev
	2	C14H13F18N15O6P3	922.0093	-0.2	-0.2	8.7	2	8.5	ok	ev
	3	C18H16F21N9O6P3	922.0096	0.1	0.1	8.8	3	4.5	ok	ev
	4	C18H15F24N3O6P3	922.0096	0.3	0.3	8.8	4	0.5	ok	ev
	3	C18H16F21N9O6P3	922.0096	0.1	0.1	8.8	3	4.5	ok	ev
	1	C12H17F17N115O6P3	922.0099	-3.6	-3.9	36.5	1	4.5	ok	ev
	3	C13H6F6N31NaO6P3	922.0127	3.2	3.4	36.5	3	24.5	ok	ev
	4	C19H9F9N23NaO6P3	922.0129	3.4	3.7	36.5	4	20.5	ok	ev
	5	C17H12F12N19NaO6P3	922.0131	3.7	4.0	36.5	5	16.5	ok	ev
	6	C19H15F15N13NaO6P3	922.0134	3.9	4.2	36.6	6	12.5	ok	ev

Automatically locate monoisotopic peak: Maximum number of formulae: 500

Check rings plus double bonds: Minimum: -0.5 Maximum: 40

Electron configuration: even

Filter H/C element ratio: Minimum H/C: 0 Maximum H/C: 3

Estimate carbon number: Generate immediately

Copy to SmartFormula Parameters Show Pattern

Results of a SmartFormula Manually operation: (a) the **SmartFormula Manually** dialog lists possible molecular formulae calculated for the selected **Meas. m/z**; (b) the **Compound Spectra** window shows the original spectrum in which the **Measured m/z** was selected (top) and the pattern for the selected formula (bottom); (c) the **SmartFormula** tab of the **Spectrum Data** window shows the results, too.

Shortcuts

Button:

Keys: **Shift+F8**