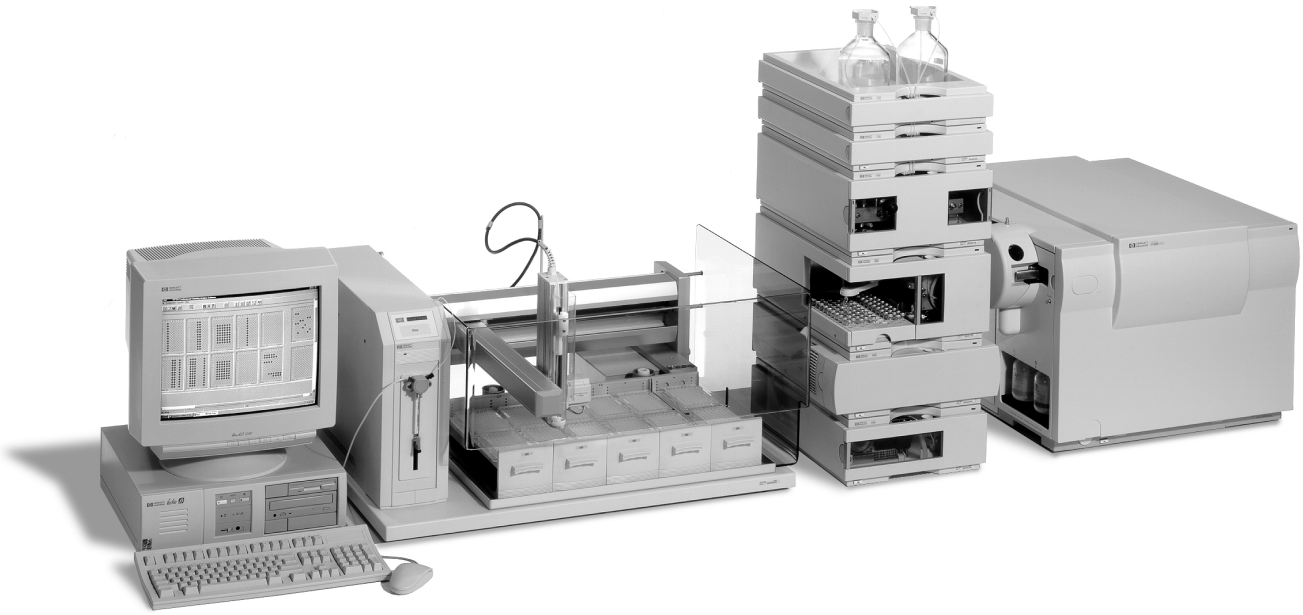
Agilent 220 micro plate sampler with Agilent 1100 Series system for flexible, high-throughput HPLC analysis

Technical Note



Abstract

The Agilent 220 micro plate sampler (MPS) is an essential part of Agilent's system for combinatorial chemistry and high-throughput HPLC analysis.

The value of the sampler is in its flexibility. It uses different sample devices and combines high sample capacity, high speed and sampling/fractionation capability in one system. The sampler, together with the Agilent 1100 Series HPLC modules and the Agilent 1100 Series mass selective detector (MSD), makes up a complete system that fulfills the requirements of combinatorial chemistry and high-throughput analysis with its robustness, ruggedness, sensitivity, selectivity and speed. The seamlessly integrated system plus the single software platform—complete with diode array and mass spectrometry detectors—simplify system setup, operation and management of large amounts of data.

Agilent Technologies
Innovating the HP Way

The Agilent 220 micro plate sampler hardware

The Agilent 220 micro plate sampler is an XYZ robot that can automate a number of liquid handling procedures such as sample injection, fraction collection and sample preparation. The standard configuration of the Agilent 220 micro plate sampler includes:

- a 100-µl syringe
- a Rheodyne® injection valve with a sample loop
- 2 needle-rinsing stations
- septum-piercing needles
- racks for up to 12 micro plates
- safety shields around the instrument and the needle

The system can be extended by the use of:

- a fraction collection accessory kit
- racks for autosampler vials and test tubes
- customized racks and plates

The high speed xyz arm moves the injector needle to the sample and fraction devices, to the injector and to the needle-rinsing stations. The syringe provides injection volumes up to 100 µl. For injection of higher sample amounts, the syringe can be exchanged. The needle, tubings and the injection port can be flushed to ensure minimum carryover. The injector with an installed loop enables the injection of a specified sample amount with high precision.

The Agilent 220 micro plate sampler can handle various types of sample plates, vials and test tubes in any combination. Frequently used rack types that hold plates, 2-ml vials and test tubes are preconfigured and can easily be selected from a screen. By default the sampler is equipped with racks for up to 12 well plates. Covers fix the sample devices or the racks on the work area. The control software also allows the programming of customized plates and racks. To design customized devices, the operator can type in the dimensions in the default rack or plate screens and save these customized sample devices with a name of the operator's choice (see figure 1). Dimensions can be entered by the nearest tenth of a millimeter.

How the Agilent 220 MPS works

Sampling and injection

During the sampling sequence, the injection valve bypasses the autosampler (load position) and connects flow from the pump to the column directly. The xyz-arm moves to the selected sample well and the needle lowers into the device. The required volume of sample is drawn into the needle by the syringe. (The volume of the loop determines the injection volume. The Agilent 220 MPS has a 5 µl loop installed. Larger volume loops can also be installed).

Then the needle lifts out of the well or vial and moves either to the wash vial to clean the outside of the needle or directly to the injector. There it lowers into the injection port. The injection valve switches to the inject position and the syringe flushes the sample into the sample loop. Then the injector switches to the inject position.

Figure 1
This shows the choice of various plates, vials and tubes which can be used.

In this position, the pump is connected to the sampler loop. All of the sample is washed out of the loop onto the column and the separation begins. If the needle wash is selected in the injector program, the needle lowers into the right wash vial to be cleaned for a preset time before it moves to the injector port. After sample injection (and during sample analysis), the valve switches back to the load position and predefined rinsing steps like injection port and needle rinse inside/outside are carried out so that the needle is always free from sample residue before the next sampling sequence begins.

Injection techniques – loop-filling modes

The injector tasks allow two different injection techniques: completely-filled sample loop or partially-filled sample loop. Which technique is used depends on the amount of sample available.

Completely filling the sample loop is the conventional method in which an excess of sample is used to overfill the sample loop. This is about 3 to 5 loop volumes of sample to achieve 95 % of the maximum loop volume. Precision for this technique is typically 0.1 to 1.0 %.

Partially filling the sample loop is used when only small quantities of sample are available. For this technique, a sandwich of air gaps and sample is injected into the sample loop. When sample without air gaps is injected into the loop, it mixes with the solvent already in the loop and some sample could be lost. Injection of sample from a partially filled loop is generally less precise (typically 0.5 to 3.0 %) than for completely filled loop injection.

Fraction collection

The optional low pressure valve installed for fraction collection is an electronically operated 3-port, 2-position valve. The valve, which is installed in the flow path between the detector cell outlet and the injector needle, is in waste position until the peak of interest is detected or a predefined collection time is reached. The software automatically changes the valve's position when the peak or fraction of interest has reached the valve and the peak is diverted through the injector needle to a fraction device. When the well is full the needle is moved to the next fraction well. The valve is switched back to waste when the peak or fraction of interest has left the needle. Each new detected peak is collected in a new well.

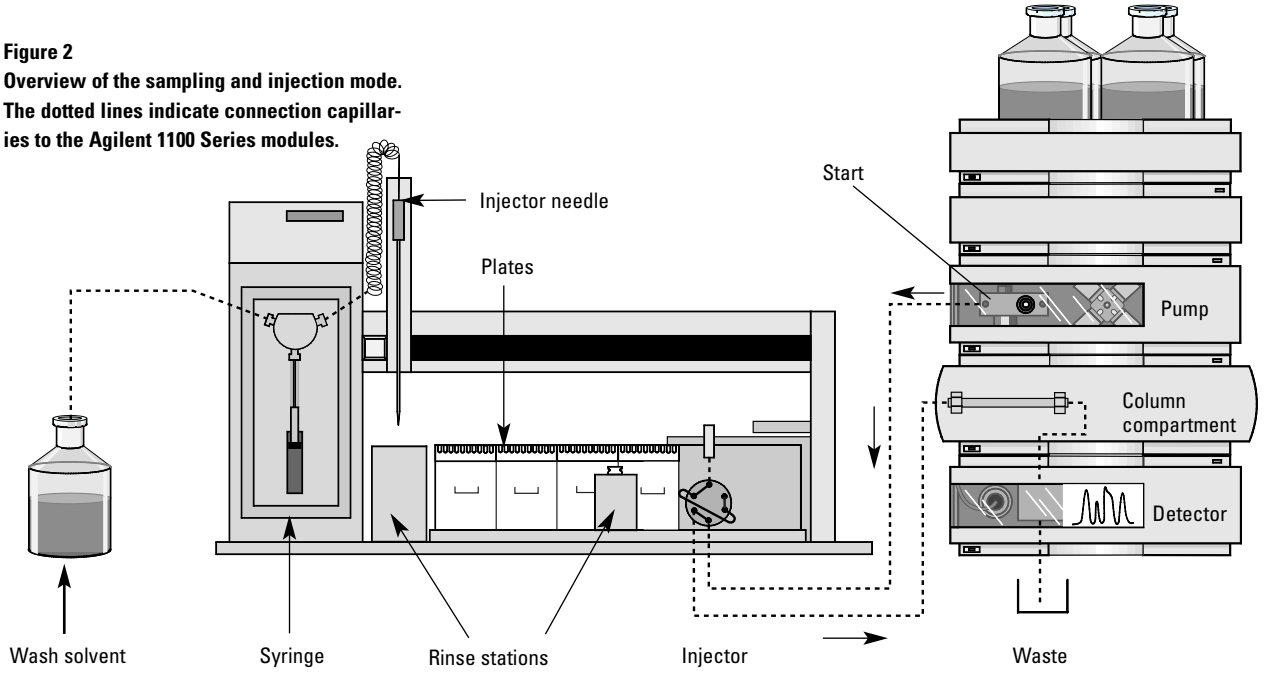
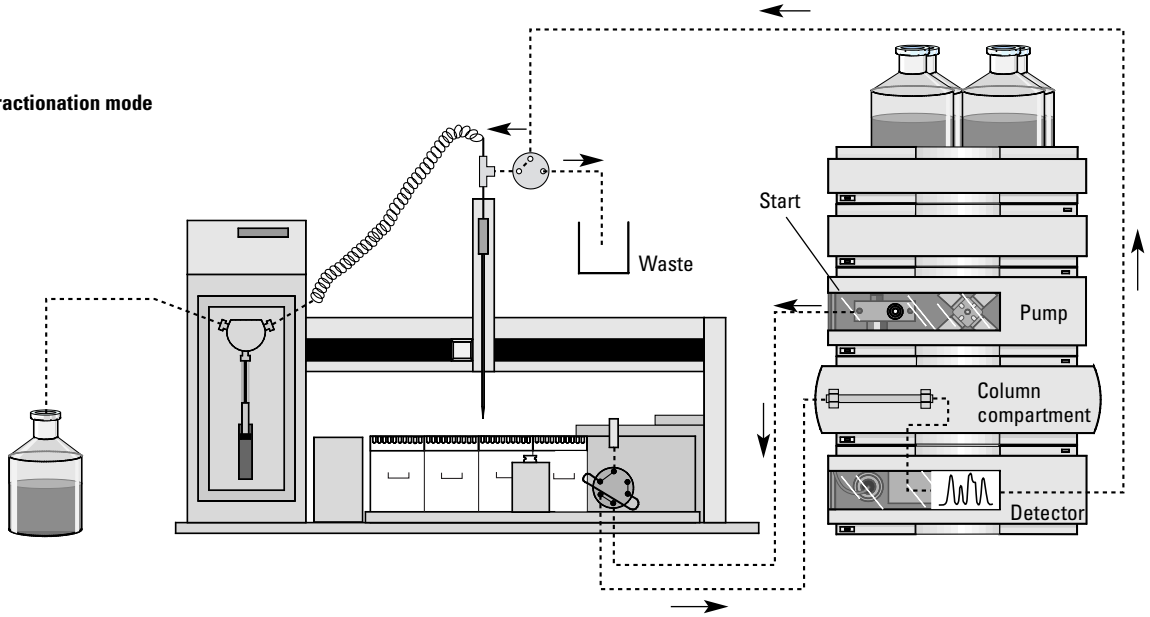


Figure 2
Overview of the sampling and injection mode. The dotted lines indicate connection capillaries to the Agilent 1100 Series modules.

Figure 3
Overview of the fractionation mode



The fraction collection mode is initiated by an Agilent 1100 Series DAD, VWD or FLD. Fractions can be collected based on peak detection or on time intervals (see figure 3).

Sample preparation

The sampler can also be used for sample preparation, for example sample derivatization and dilution. For this application, up to two well plates or racks for other sample devices on the sampler can be selected as sources. The program allows drawing sample from a sample plate and drawing diluent or reagent from the sources. The needle content is then ejected in a destination plate or rack, where it can be mixed. This procedure can be repeated for the next sample(s) or a specified volume can be drawn and injected onto the column for analysis.

How to reduce carryover

The main contribution to carryover comes from sample on the outside of the needle. Washing the needle after drawing the sample removes the sample from the surface of the needle immediately. A rinsing station and a wash vial help to reduce carryover to an absolute minimum.

Control of the Agilent 220 MPS

The sampler is controlled by software (CC-Mode) integrated into the Agilent ChemStation. This software allows:

- sample selection and automated study generation
- selection of fractions for analysis
- review of sample results
- confirmation of the presence of specific compounds based on MS signal
- specification of purity requirements for quantification and purity calculation
- graphical representation of the results
- printing of sample and fraction data
- import/export for data exchange with other applications like Microsoft® Excel®
- customization of well plates and other sample devices

What is a study

A study in CC-Mode can be compared with a sequence in the Agilent ChemStation. It is a series of instructions that automates the analysis, data evaluation and reporting of samples. A study contains at least one analytical method that comprises all the parameters for acquisition and data evaluation, including integration and reporting. The system can be set up to acquire data from a number of samples by different methods.

A study includes:

- a list of samples
- a list of fractions
- the sampler configuration
- analytical and sampler methods
- reporting options
- purity calculation parameters
- the data file structure

All integration parameters can be set within the analytical method.

The complete information is stored in a database and can be retrieved later for sample review and reprocessing.

The study layout screen

When the software is started, the main screen showing the *Study Layout screen*, the *Menu Bar* items, the *Tool Bar* items and the *Status Bar* pops up, as shown in figure 4.

This user interface shows the system status, the study layout of the well plates on the sampler, and an overview of the sample and fraction position. It also provides tool bar and menu bar items for selecting study lists, study parameters, sampler parameters, purity calculation and report options.

This screen is used to build a study by selecting samples with a mouse click from the graphical user interface either randomly, by row and column, or by dragging. It is also possible to inject collected samples from fraction plates for re-analysis. The screen also shows the number of plates that is being used for samples (red), fractions (blue), or sample preparation (gray).

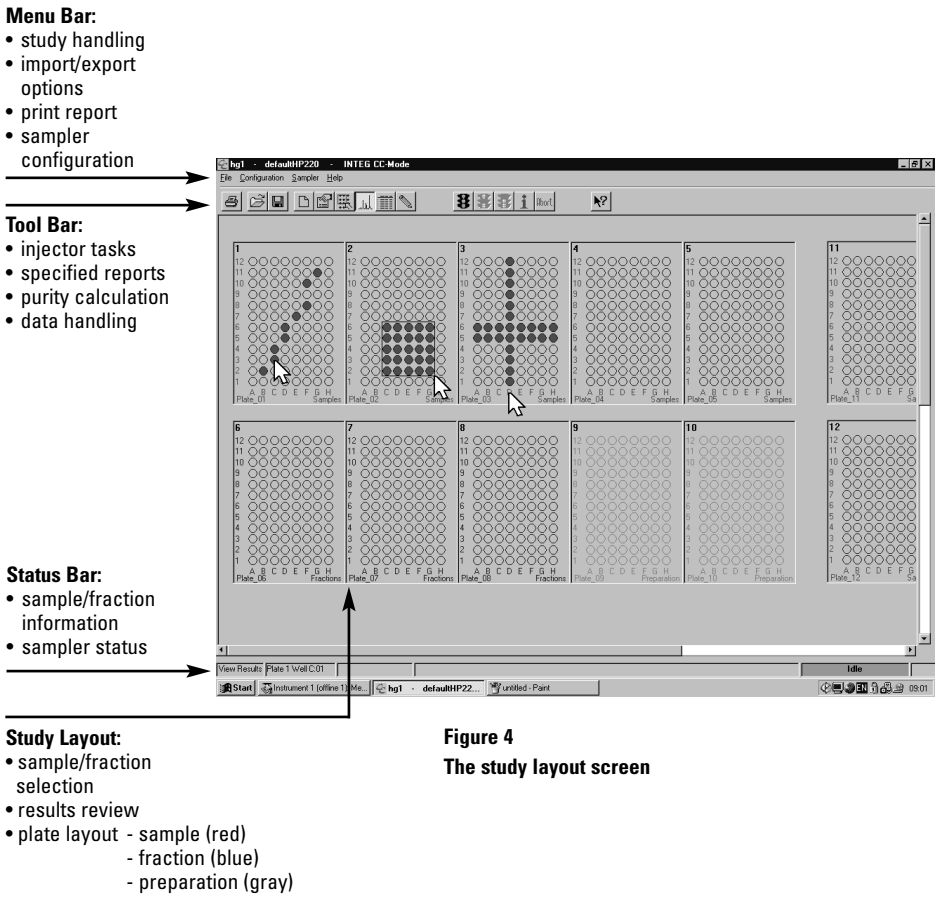


Figure 4
The study layout screen

Study lists, samples and fractions

Once samples in the study layout have been added to a study, a list of all selected samples is automatically created, as shown in figure 5. This information includes sample location, sample name, and Agilent ChemStation method(s) used for sample analysis. A double click on the selected sample from the list shows the sample information. This dialog box can be used to change the sample name and/or the methods for each sample or to add new methods to use for the analysis of the selected sample.

When fractions are collected, the fractions list shows:
- the number of injections
- the peak within the run (*Peak #*)
- the location where the fraction begins (*StartLoc*)
- the number of wells this fraction occupies (*# Wells*)
- the run time at which fraction collection started and ended (*StartTime* and *EndTime*)

The expected masses screen is where you specify the monoisotopic mass of the target compound(s) for each sample. You can type the mass or type the molecular formula and let the software calculate the mass for you. This information, in addition to other sample information such as well position, the sample name and the Agilent ChemStation method, may also be imported as a comma separated value (CSV) file.

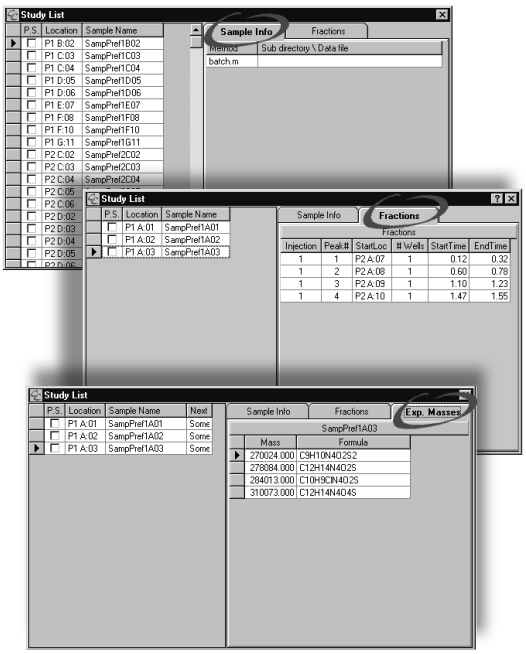


Figure 5
The study list screens show information about samples, fractions and expected masses.

The study parameters screen

The study parameters screen, as shown in figure 6, enables the selection of the parameters necessary to run the sample analysis and to generate reports. The study parameters screen provides access to the following tasks:

- Agilent ChemStation method for sample analysis
- conditional logic to method execution (optional)
- Agilent 220 MPS sampler tasks
- purity calculations (optional)
- study layout
- sample name format
- data file path
- report options

From this screen the Agilent ChemStation method(s) for sample analysis and processing can be selected.

A maximum of nine methods can be linked to run on the same set of samples. The selected method(s) will be used for all subsequent samples added to a study.

The software adds conditional logic to method execution. When multiple methods are selected, the operator can choose under which conditions the next method will be run:

- if no target masses are found,
- if some target masses are found,
- or if all target masses are found.

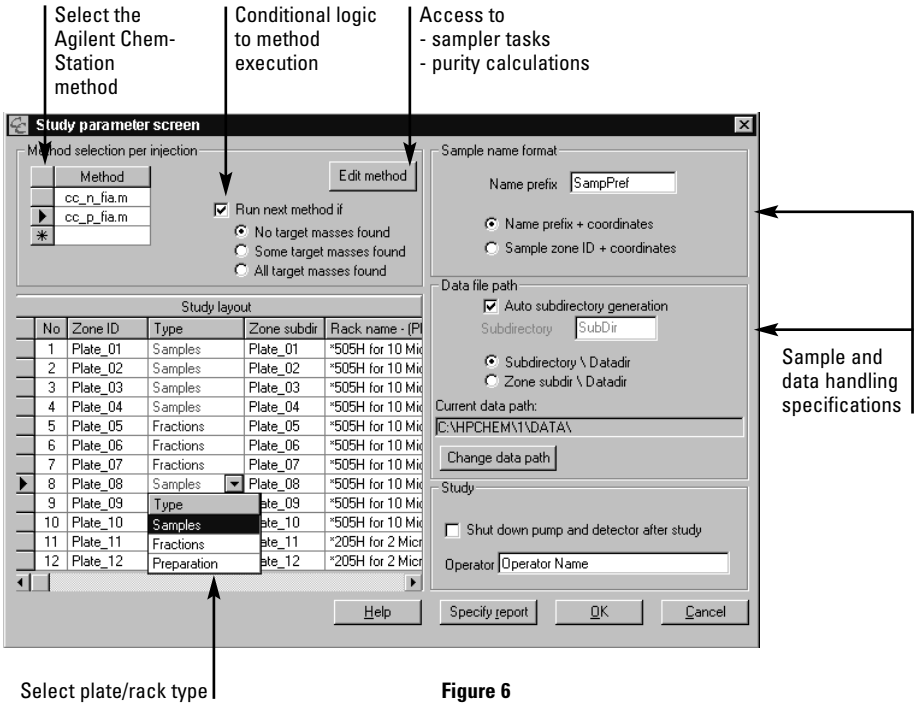


Figure 6
The study parameter screen

For example, some users screen wells quickly using a flow injection method and only switch on to a chromatography method if the target mass is found above a specified level. This can save time and increase throughput. If the "*run next method*" box is not checked, all methods will be run. In order to be "*found*", the target masses must be present in high enough concentration to meet the qualification criteria the user has set.

The study layout shows the application for which the plates and racks on the sampler are currently used (samples = red, fractions = blue, preparation = gray). The selected plate type is shown in the study layout screen with the appropriate color.

Sample Name format and the *Data File Path* is selected in the right part of the study parameters screen.

The screen also gives access to the sampler parameters screen from which the sampler tasks can be selected.

The CC-Mode software offers a number of report options such as multi plot, extended plate report and UV-chromatogram, mass spectrum and total ion chromatogram (TIC).

The sampler parameters screen

In this screen, the operator selects all the tasks necessary to prepare and analyze the sample (see figure 7). The tasks have to be selected from a list to create an injector program. The required parameters for each task can be entered separately. The tasks will be executed in the order in which they appear in the *Task List*.

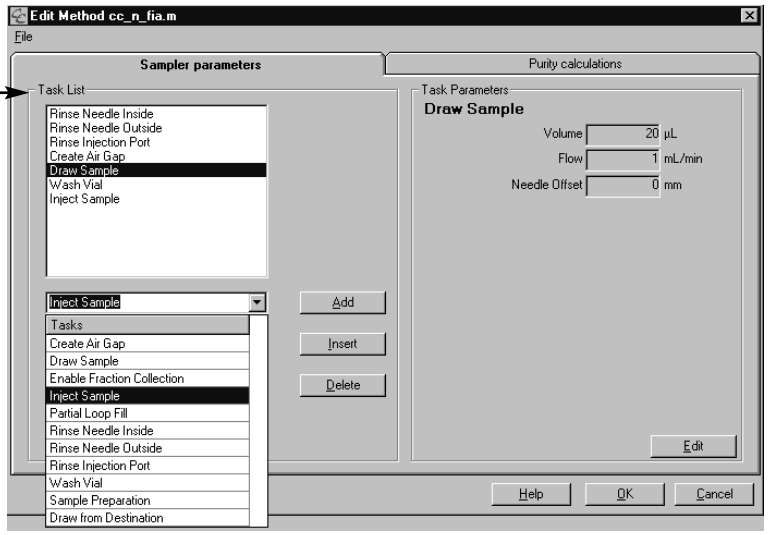


Figure 7
The sampler parameters screen

Description of the tasks:

Create air gap	To keep sample separated from liquids in the needle/tubing, an air gap should be included before drawing the sample.
Draw sample	The specified sample volume is drawn from the sample device. A needle offset can be selected which allows the drawing of a sample from a defined liquid level.
Enable fraction collection	1. Collect fractions based on peak detection. Three parameters determine the duration and moment of collection: threshold, peak slope and maximum fraction duration. 2. Collect fractions based on fixed times. In this mode fraction collection starts at the first Start Time and ends at the End Time. This is repeated for each line in the time table; up to 29 collection windows can be programmed per run (method).
Inject sample	This command moves the needle into the injection port, ejects the sample into the sample loop, and rotates the injection valve to the inject position. The pump flow flushes the sample onto the HPLC column.
Partial loop fill	This method is used when only small quantities of sample are available. Using this technique, a sandwich of air gaps and sample is ejected into the sample loop. This method is generally less precise than for completely filled loop injection.
Rinse needle/ injection port/wash vial	These tasks are used to wash the needle inside and outside, for cleaning the injection port and for an additional needle wash step after the sample is drawn and before sample injection to reduce carryover to a minimum.
Sample preparation	This command is used for sample derivatization or dilution.
Draw from destination	The command is used to draw and inject a specified sample volume from a destination plate.

Sample preparation

This command allows designation of up to two plates or racks (*Source Zone 1* and *2*) for diluent or reagent for sample preparation such as derivatization or dilution. This command allows use of the Agilent 220 MPS as a dilutor without sample injection, as shown in figure 8.

After the desired sample is drawn, the needle moves to *Source Zone 1* and *2* and draws a specified volume of diluent or reagent. Then the needle moves to the first free position in the *Destination Zone* and the total volume (sample, source 1 and source 2) is ejected. The total ejected volume is now mixed in the destination as specified by *Mix Strokes*. The mixed sample can now be injected onto the column using the *draw from destination* command or another sample can be prepared as described above.

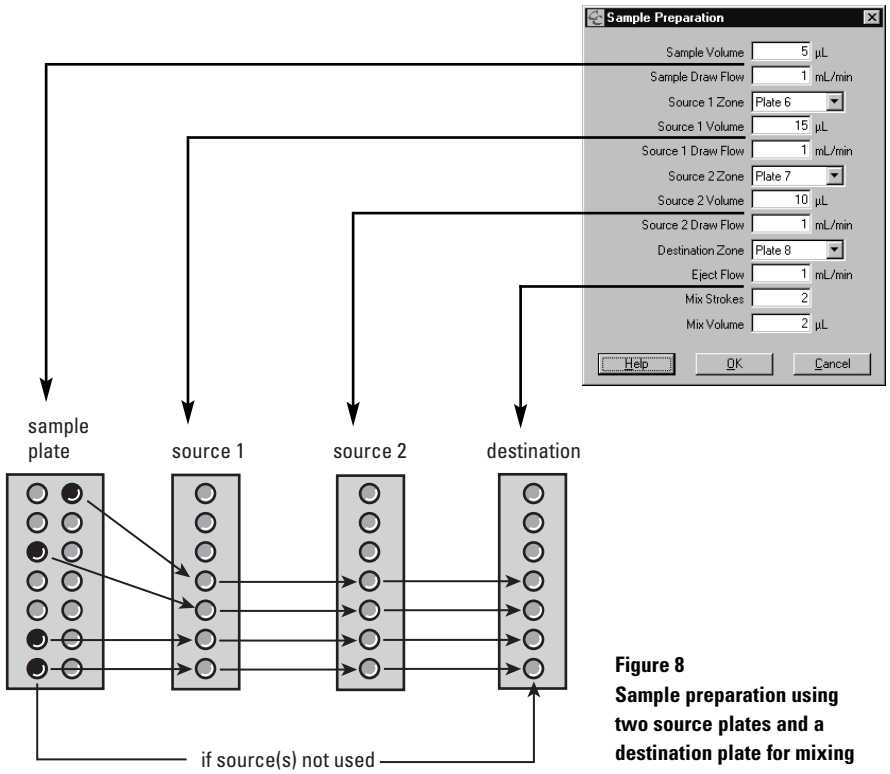


Figure 8
Sample preparation using two source plates and a destination plate for mixing

Start/reprocess a study

The software can run whole studies or partial studies, analyze fractions and reprocess whole studies or partial studies.

The status information screen

The Status screen displays information about what is happening with the Agilent ChemStation and the Agilent 220 MPS while the system is running. This includes which sample from the study is being analyzed, the study name, the plate/rack name, the well position and the corresponding data file name.

Result review – samples and fractions

The main window of CC-Mode shows a diagram of all configured plates and racks. After a study has been run, all sample and fraction wells will be color-coded. The sample wells are red, the fraction wells are coded with alternate light green and dark green colors. Adjacent wells that belong to the same peak will have the same color, for example light green. The next peak will have wells colored with dark green, and so on.

The procedure to view samples, fractions and the corresponding chromatogram is to click single wells and the relation between samples and fractions (if available) is made visible, as shown in figure 9.

Import/export of data

The software allows the user to import information such as sample name, Agilent ChemStation method and expected masses from a CSV file using applications like Microsoft Excel. The spreadsheet is set up with the information for each sample and saved as a CSV file, as shown in figure 10.

In the import options screen the names of the fields you wish to import are moved from Available fields to Fields in CSV File in the order in which they occur in the CSV file (figure 11).

Reports can also be exported to CSV files.

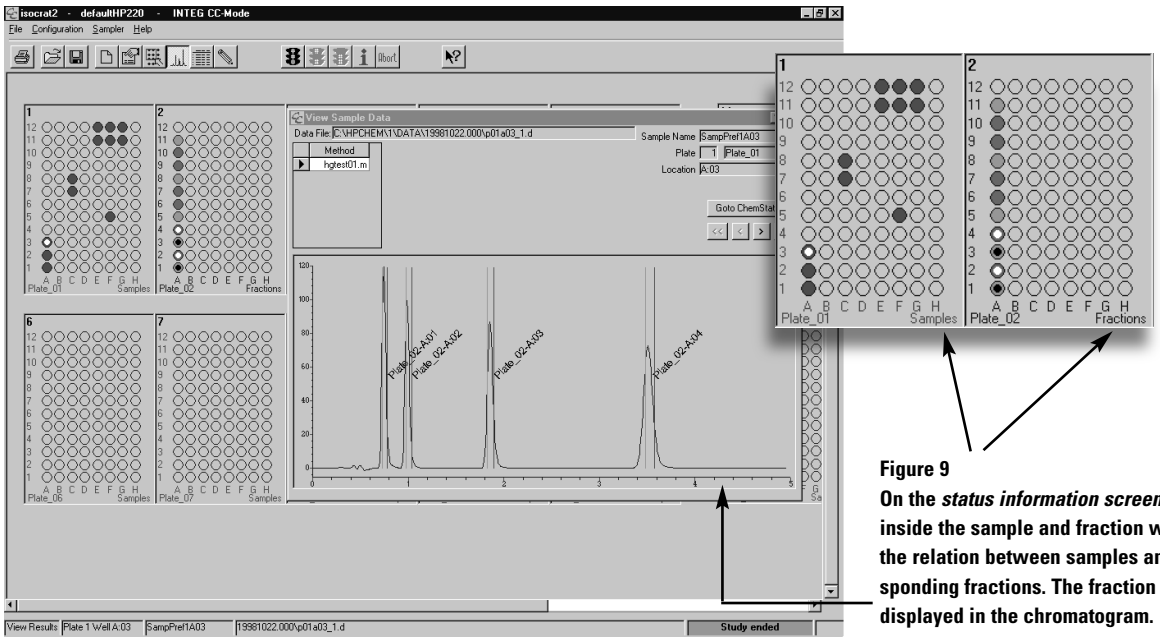


Figure 9
On the *status information screen* a dot appears inside the sample and fraction well to indicate the relation between samples and the corresponding fractions. The fraction information is displayed in the chromatogram.

Microsoft Excel - import

File Edit View Insert Format Tools Data Window Help

	A	B	C	D	E
1	well position	sample name	notebook #	anal.method	exp. masses
2	a:1	FI_A01	256-071	cc_fipos.m,cc_fineg.m	270,278,284,310
3	a:2	FI_A02	256-072	cc_fipos.m,cc_fineg.m	C10H9ClN4O2S
4	d:1	FI_D01	256-073	cc_fipos.m,cc_fineg.m	270,278,284,310
5	d:2	FI_D02	256-074	cc_fipos.m,cc_fineg.m	C10H9ClN4O2S
6	g:1	FI_G01	256-075	cc_fipos.m,cc_fineg.m	270,278,284,310
7					

Figure 10
Part of an Excel spreadsheet

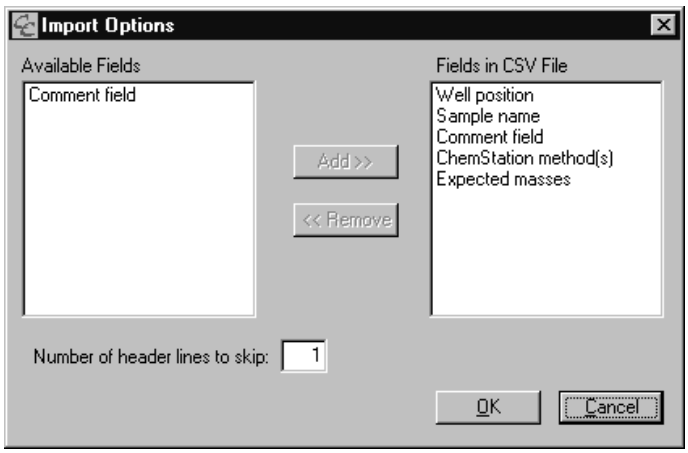


Figure 11
Easy adding or removing of import options

Data analysis software

The optional data analysis software allows the operator to set up the system to confirm the presence of specific compounds. The user can specify purity requirements for qualification, select other purity calculations, graphically display the results, and generate printed reports.

The purity calculations screen

The purity calculations screen (figure 12) allows you to specify the calculations to be used to determine percent purity. This information, like that in the sampler parameters screen, is stored with the Agilent ChemStation method.

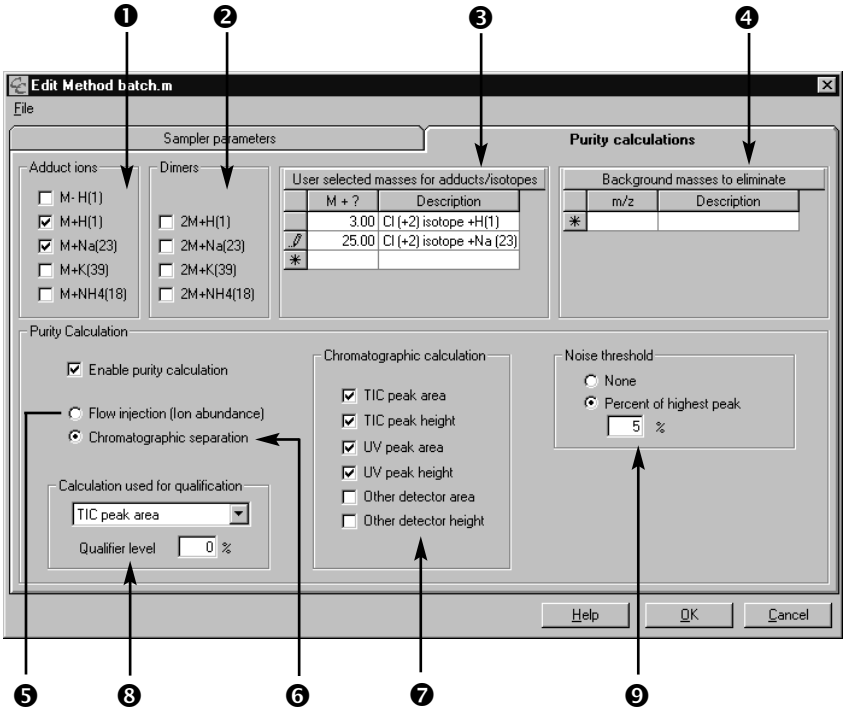


Figure 12
Purity calculations screen

You can specify:
❶ adduct ions
❷ dimers
❸ user-selected masses for adducts/isotopes
❹ background masses to eliminate

In the top part of the purity calculations screen you can tell the system which ions might be formed for your target compound. The expected mass is the mono-isotopic mass of a compound. The LC/MSD looks at ions. In atmospheric pressure ionization, several kinds of ions can be formed from the sample molecule, depending on the analytical method and ionization mode. All adduct, dimer, and isotope ions specified in this section will be counted as belonging to the expected mass; all other ions (above the noise threshold) will be added as impurities. You can also tell the system which of the ions present in the sample do not belong to your target compound. This will keep these background ions from being added as impurities.

Specify actual calculations in the bottom part of the screen:
- flow injection (ion abundance)
- chromatographic separation
- chromatographic calculations
- qualifier level
- noise threshold

When you select *Flow injection* (❺), the individual compounds in the sample are not separated. Therefore the purity calculation is done by using the ion abundance of an averaged mass spectrum. This type of calculation is appropriate only for mass spectral data.

When you select *Chromatographic separation* (❻), you must specify what signals the system should use for purity calculations in the chromatographic calculations section. You must also select the calculation used for qualification, and set the qualifier level (percent purity).

In the *Chromatographic calculations area* (❼) you select which calculations will be performed on chromatographic data. From these calculations, you must select one to be used for qualification.

The *Qualifier level* (❽) is the percent purity that the selected signal must exceed in order to be considered present in the sample ("found"). You need to adjust the qualifier level to reflect the number of compounds and the relative amount of each compound expected.

In *FIA, the Noise threshold parameter* (❾) is calculated as the percentage of the most abundant ion; for chromatography, the noise threshold is calculated as the percentage of the signal selected for the qualifier.

Purity calculations

For all purity calculations, all signals below the noise threshold are ignored. That is, only signals above the noise threshold are used in the calculations.

Flow injection

Taking the averaged spectrum across the largest peak in the TIC at half-height, the purity is calculated by summing the abundances of all the selected ions and dividing this sum by the sum of all the ions. This ratio is multiplied by 100 for percent purity.

Chromatographic separation

All chromatographic peaks are first integrated. The purity calculation depends on whether peak area or peak height is selected. For mass spectral data, an extracted ion chromatogram (EIC) for each selected ion is generated. The sum of the integrated peak height or area in the EIC for each selected ion is divided by the sum of the TIC. This ratio is multiplied by 100 for percent purity.

For UV or "other" data, the software uses an EIC for each target mass to locate each peak of interest. The area or height of the peak of interest is divided by the sum of the peaks in the chromatogram. This ratio is multiplied by 100 for percent purity.

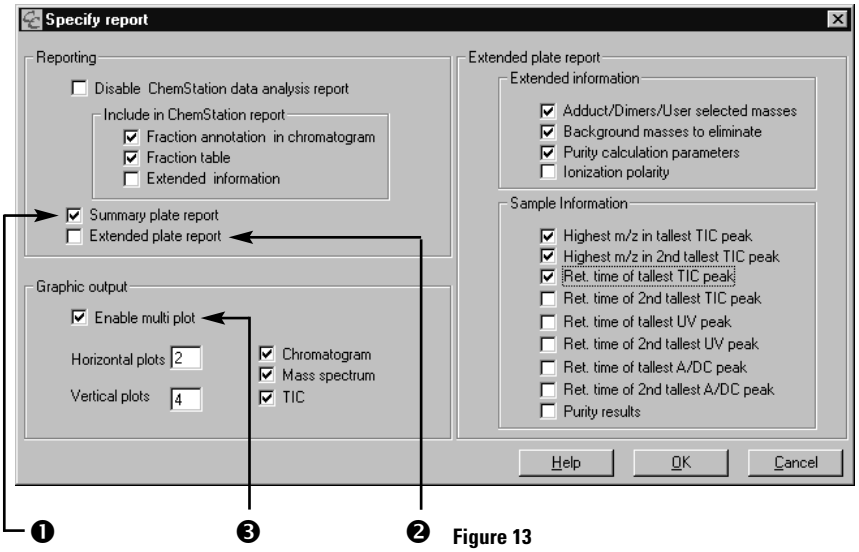


Figure 13
The *Specify report screen* allows you to select the options for an extented plate report, as shown in figure 15

The specify report screen

The specify report screen allows you to set up a variety of printed reports, as shown in figure 13.

The *Summary plate report*(❶) is useful for quick screening applications and for printing a simple graphical report that shows the qualification result of each sample well. Each method for each plate is printed on a separate page.

The *Extended plate report* (❷) prints all the selected information for each sample and each method. Only results from the extended report can be exported to a CSV file.

The *Multi Plot option*(❸) enables graphical printing, and can give multiple plots on one page. Three kinds of information can be plotted graphically: UV chromatogram, Mass spectrum (chromatographic data or FIA data), or TIC.

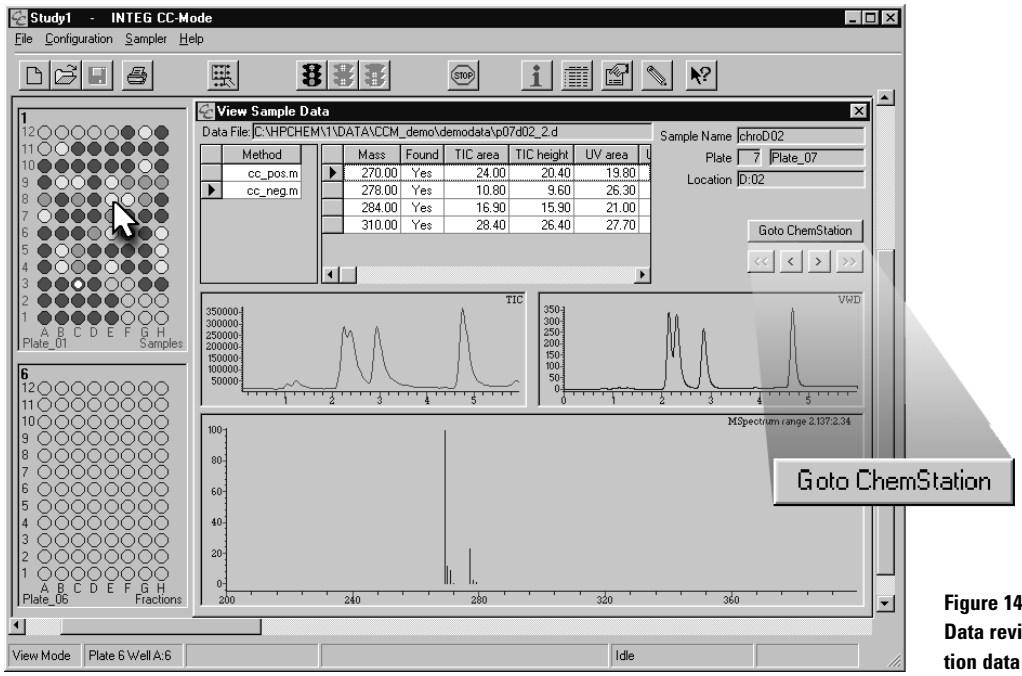


Figure 14
Data review with the Agilent ChemStation data analysis feature

Data analysis results

The study layout screen shows a diagram of all configured plates and wells, as shown in figure 14. After a study has been run, all sample wells will be color-coded. The colors show the results of the last method run on each well:

- Red: no target masses found
- Yellow: at least one of the target masses found
- Green: all target masses found

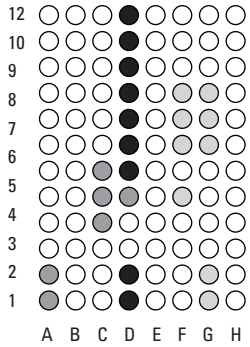
The sample data window is viewed by clicking on a well. The data for the selected method is displayed; to view data from another method, click the box next to the left of the method name to select it. The chromatograms and spectra are displayed almost instantaneously for very fast review of a study.

For a more in-depth data review, the *Goto ChemStation* button loads the appropriate file in the Agilent ChemStation data analysis. Here extensive data review is possible, including generation of MS spectra from any part of the TIC. For those wanting to do data review on their desktop PC, this is also possible with a local copy of the CC-Mode and Agilent ChemStation software that give access to the study data.

Extended plate report
Plate number: 7
Plate name: Plate_07

Study name: Testdemo
Study date: 1/6/99 3:46:12 PM
Print date: 1/12/99 10:25:49 AM

Plate score: % Red 33.3 % Yellow 33.3 % Green 33.3
Plate score and graphics refelct the state after the last method run.



Method: cc_pos.m
Ionization polarity: Positive
Purity based on: TIC peak area Qualifier level: 1

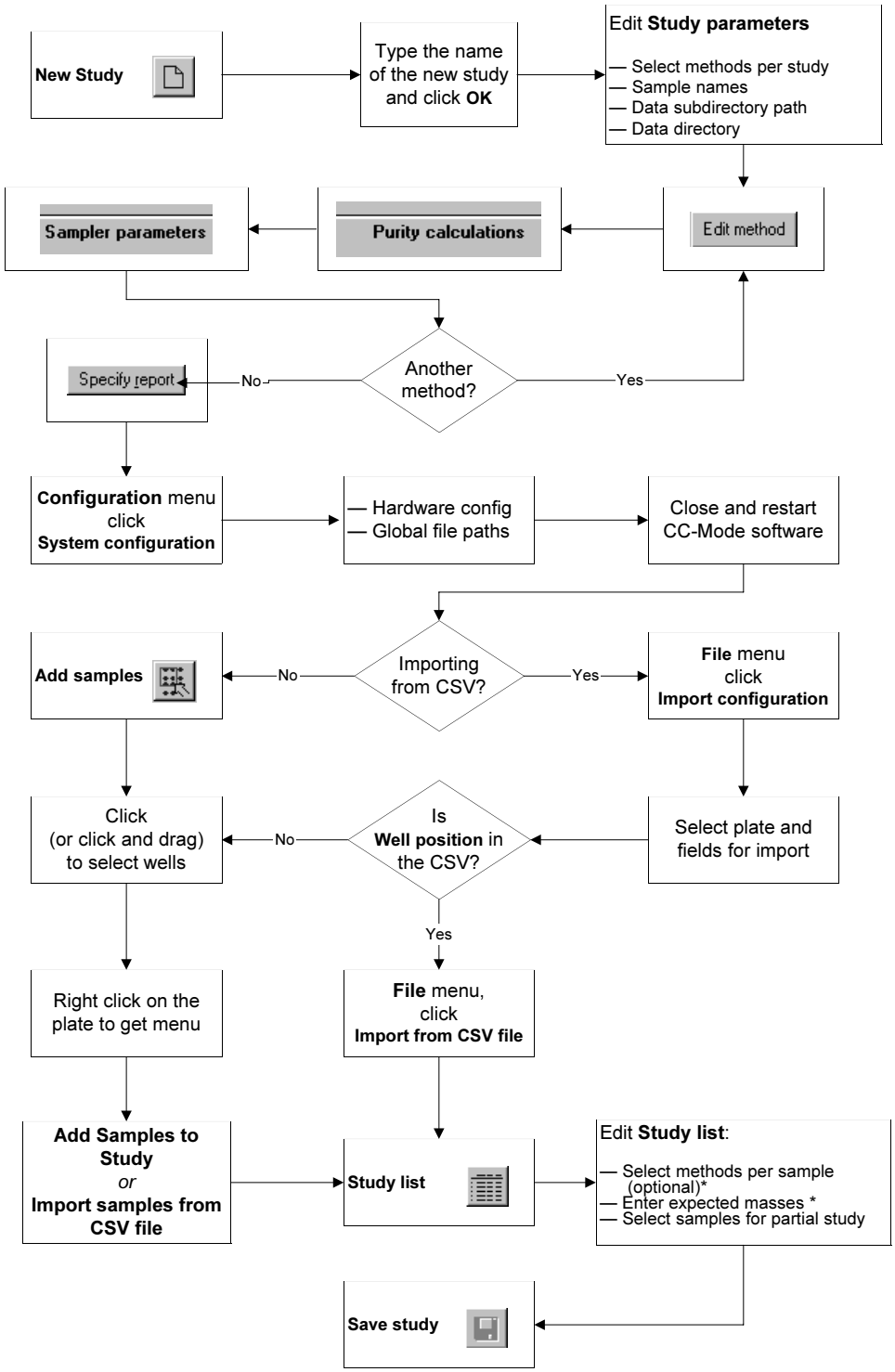
Items used in Purity Calculations:

Adducts	Dimers	User Defined Adducts
M+H(1)	<none>	2 M+2

Well Nbr	Mass	Found	%Purity by TIC Pk Area	%Purity by TIC Pk Height	%Purity by UV Pk Area	%Purity by UV Pk Height	Highest m/z in tallest TIC peak	Highest m/z 2nd tallest TIC peak	RT of tallest TIC Pk	RT of 2nd tallest TIC Pk	RT of tallest UV Pk	RT of 2nd tallest UV Pk
P7 A:01	270.00	No	0.0	0.0	0.0	0.0	399.20	399.05	5.67	3.22	3.11	–
	278.00	No	0.0	0.0	0.0	0.0						
	284.00	Yes	7.11	6.75	100.0	100.0						
	310.00	No	0.0	0.0	0.0	0.0						
P7 A:02	270.00	No	0.0	0.0	0.0	0.0	400.00	399.85	5.61	5.41	–	–
	278.00	No	0.0	0.0	0.0	0.0						
	284.00	Yes	2.7	3.03	0.0	0.0						
	310.00	No	0.59	0.81	0.0	0.0						
P7 D:01	270.00	Yes	12.46	15.59	15.56	23.9	333.05	301.00	4.77	2.41	4.68	2.31
	278.00	Yes	31.17	31.12	23.59	24.62						
	284.00	Yes	8.74	9.54	18.6	19.55						
	310.00	Yes	38.59	44.3	42.25	31.93						
P7 D:02	270.00	Yes	11.71	15.93	15.9	24.01	333.05	301.00	4.78	2.42	4.68	2.31
	278.00	Yes	28.07	29.96	23.63	24.54						
	284.00	Yes	7.55	8.8	18.67	19.58						
	310.00	Yes	35.32	44.25	41.8	31.87						
P7 G:01	270.00	No	0.47	0.58	61.23	82.25	309.00	395.35	2.97	4.80	2.87	5.45
	278.00	Yes	1.17	1.2	61.23	82.25						
	284.00	Yes	54.11	53.49	61.23	82.25						
	310.00	Yes	1.51	2.3	11.52	5.1						
P7 G:02	270.00	No	0.0	0.0	0.0	0.0	309.00	399.90	2.96	5.91	2.87	5.81
	278.00	No	0.0	0.0	0.0	0.0						
	284.00	Yes	57.87	59.67	65.23	85.33						
	310.00	No	0.2	0.3	65.23	85.33						

Figure 15
Printout of the extended plate report as specified in figure 12

This flowchart of the software operation shows how to set up a new study in CC-Mode



* Skip these steps if the information is contained in the imported CSV file.

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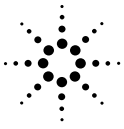

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