

# on-line

FOR MILLENNIUM SYSTEM USERS

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MILLIPORE

Waters Chromatography

Welcome to **on-line**...

a newsletter designed specifically for you,  
the **Millennium™** System user.

**on-line** is intended to be a resource designed to help you take full advantage of the power of the Millennium technology by creating a worldwide network of Millennium System users and Millipore technical specialists.

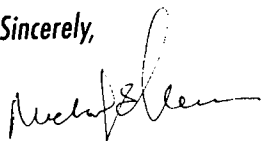
Each issue will contain articles that will provide insight into the many features and applications of the Millennium 2010 Chromatography Manager and the Millennium 2020C/S Networks.

In addition, our worldwide support group will answer your questions about Millennium Systems. We'll round off each issue with brief helpful hints, training opportunities, descriptions of coming enhancements and, finally, offer you a chance to get involved.

We want to hear from you. Please let us know what kinds of articles and topics interest you, what applications you find most valuable and how you feel about your Millennium System.

Together we can access the true potential of the Millennium technology: the power to transform your world without turning it upside down. Imagine the possibilities.

Sincerely,



Michael E. Leeman  
Senior Vice President  
Worldwide Marketing

# Internal/External Standard Quantitation

by: B. Citron, S. Harrington and I. Neitring

HPLC is an established standard analytical tool used in the quantitative analysis of a broad range of samples and sample matrices. Major elements of HPLC methods – mobile phase composition, detector operating parameters, column and sample preparation vary considerably and continue to be topics of debate.

A standard calibration routine and sample analysis sequence resides within any analytical method. It is early in the methods devel-

a single point calibration response (also referred to as single level), the standard concentration is not intentionally varied. However, multiple weighings of a standard are frequently prepared to eliminate the possibility of errors causing incorrect responses. In the case of multi-point standard calibration (also referred to as multilevel), you intentionally vary the concentration of the standards, usually to encompass the expected concentration range of the samples.

When working with a HPLC chromatography data system, it is essential that you be able to set up the standard calibration routines in a convenient and systematic manner, and that the results can be easily reviewed and printed when required. The Millennium™ 2010 Chromatography Manager puts all these necessary features in Review window (See Figure 1). What follows is a discussion of how to perform internal or external standard quantitation using the Millennium Chromatography Manager.

## Quantitation by the Method of External Standard

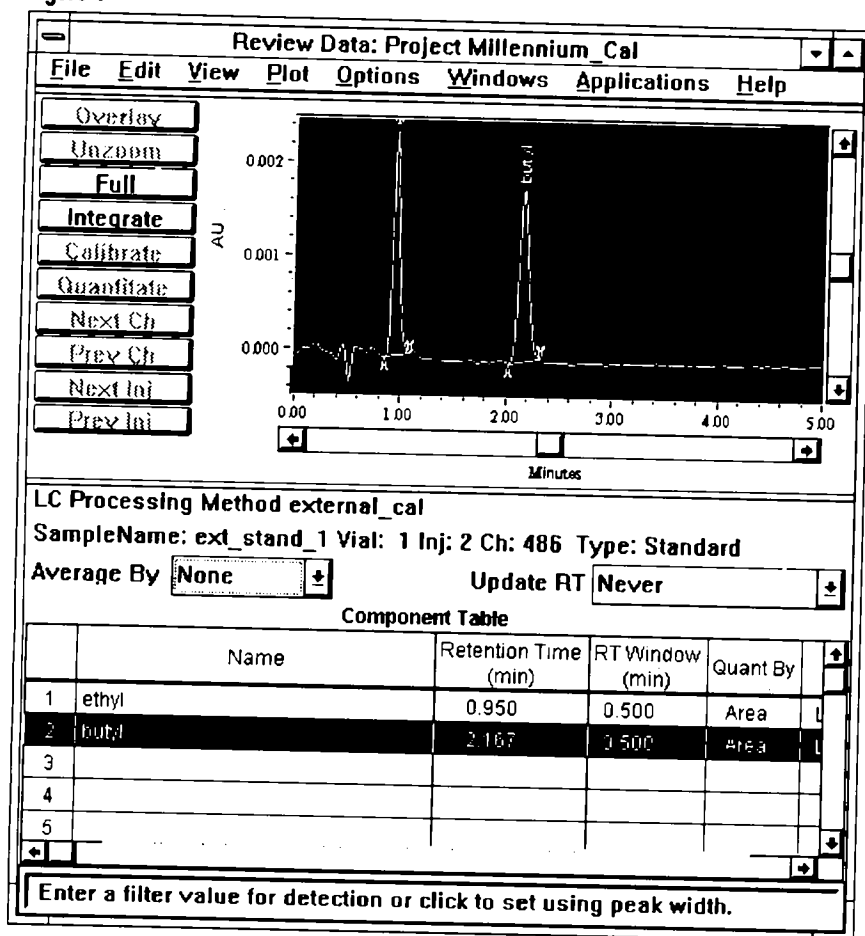
After the standard is injected and the data acquired, the data file is called into Review. You then develop a Processing Method in Review which establishes the exact integration parameters for accurate peak integration and labels the standard peaks. This is done by selecting either View, Integration Table or View, Component Table in Review.

Once you are satisfied with the results of the newly created processing method, the processing method is saved and linked to a Method Set. The Method Set defines the Instrument Method, the Processing Method and the Report Method used to obtain, process and report HPLC results.

Once the Method Set is established, you define the sequence of standard and sample injections by filling in the Sample Loading Table of the Quickset Control window (Figure 2). You will notice that the Component Loading Table can be reviewed and/or edited while still viewing the Sample Loading Table. For establishing a multilevel calibration routine, this feature is invaluable.

Within the Sample Loading Table there is a Function column that establishes the injection as a standard or sample and links that injection

Figure 1



opment stage that you must determine whether a method of external or internal standard quantitation will be used.

In addition to standard calibration, you are faced with the decision to generate either a single point or multipoint calibration curve to determine the standard response for use in the analysis of unknowns. In the case of generating

tion to the Component Loading Table. The user simply chooses Edit, Components within Quickset Control and edits the components for each standard line in the Sample Loading Table, entering the name of the peak and the amount this standard represents.

A feature used to reduce manual entry of standard data is available through a special function called Copy Components from Method, accessible from the Edit, Components window (Figure 2). This fills in the name of the components for the standard using the name of the peaks previously identified in Review and requires you to enter only the amounts for the standard peaks representing the current level.

In the case where multiple standards of varying concentrations are used, you can use the function Copy Components from Previous Sample to enter the same component information for all remaining standards in the Sample Loading Table. You only need to update the Amount column as you complete the editing of standards.

When the Run Mode from Quickset Control is either Run & Process or Run & Report, the calibration curves will be generated automatically (Figure 3). In the Calibration Curve Review window, each standards calibration response and the resulting statistical data calculated from the calibration curve (for multilevel calibration routines) are visible. In Figure 3, the calibration curve was generated for five different levels for the component ethyl, eluting at 0.950 minutes.

## Quantitation by the Method of Internal Standard

The Millennium Chromatography Manager provides the power and simplicity to set up single or multilevel calibration routines quickly. The same steps described above are used to quantitate by an internal standard as well. As before, the standard solution is injected, but this time the internal standard component is also identified in the Component Table in Review. In Figure 4, the internal standard peak is labeled Intstd. For each remaining component, you must enter the same name in the Int Std column of the Component Table as you used to identify the internal standard. By selecting Area in the Quant By column for the internal standard, peak areas will be used instead of peak heights to determine amounts in the method.

Figure 2

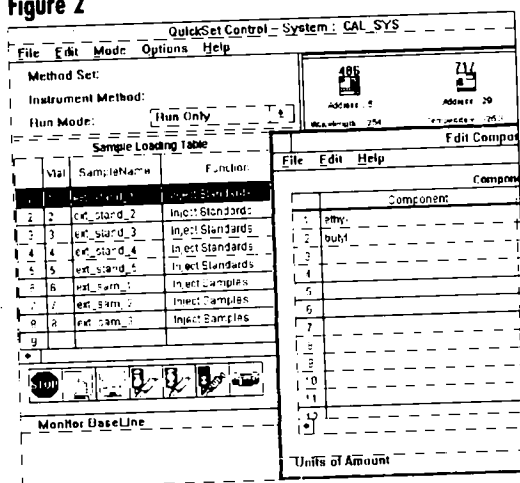


Figure 3

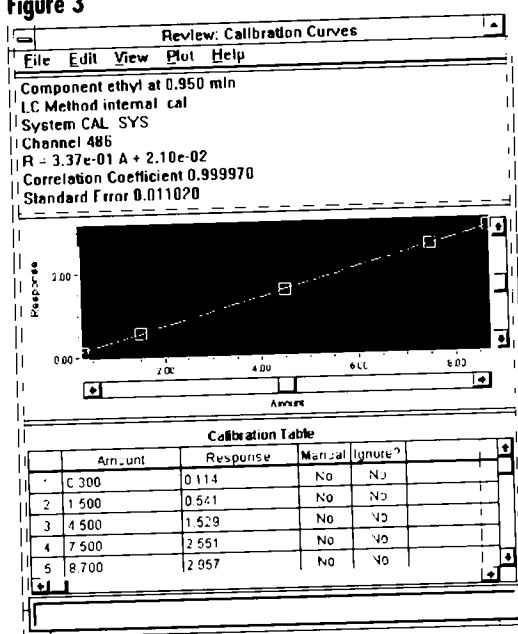
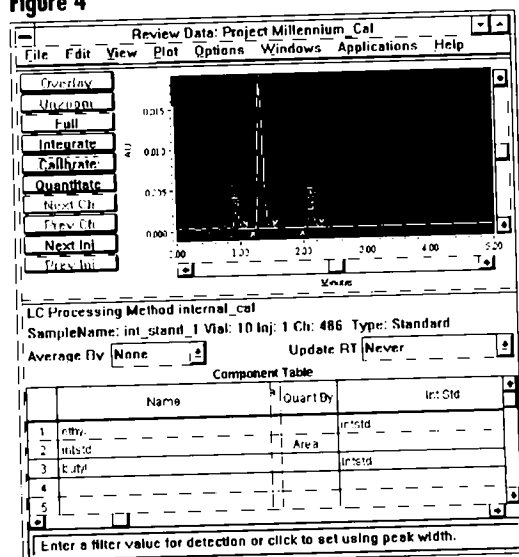


Figure 4



Millipore offers training on Millennium Systems on the following dates and locations (call your local subsidiary for further details including cost information):

### MARLBOROUGH, MASSACHUSETTS

Millennium 2010  
Chromatography  
Manager Seminars:  
February 16 - 19  
May 18 - 21  
June 22 - 25  
September 20 - 23  
November 15 - 18

Millennium 2010  
Chromatography Manager/  
996 PDA Seminars:  
May 24 - 27  
July 27 - 30  
October 19 - 22

Contact: Customer  
Education Coordinator  
Tel: 508-624-8502  
Fax: 508-624-8892

### FRANCE

#### ST. QUENTIN

Millennium 2010  
Chromatography Manager  
Seminars:  
May 11 - 13  
June 8 - 10  
September 21 - 23  
November 23 - 25

### LYON

Millennium 2010  
Chromatography Manager  
Seminars:  
March 23 - 25

### MOLSHEIM

October 12 - 14  
Contact: Jean Vialas  
Tel: (1) 30 12 7000  
Fax: (1) 30 12 7180

### ESCHBORN, GERMANY

Millennium 2010  
Chromatography Manager  
Seminars:  
March 9 - 11  
June 15 - 17  
September 7 - 9  
November 30 - December 2

Continued on page 4

## ESCHBORN, GERMANY

Millennium 2010 Chromatography  
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996 PDA Seminars:  
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June 22 - 24  
December 7 - 9

Contact: Reinhard Strauss  
Tel: (6196) 4940  
Fax: (6196) 482388

## SPAIN

### MADRID

Millennium 2010 Chromatography  
Manager Seminars:  
February 10 & 11  
June 2 & 3  
September 29 & 30

## BARCELONA

Millennium 2010 Chromatography  
Manager Seminars:  
February 17 & 18  
October 6 & 7

Contact: Ramon Figueras  
Tel: (93) 3259616  
Fax: (93) 3259896

## COPENHAGEN, DENMARK

Millennium Chromatography  
Manager Basic Training:  
March 12  
June 18  
October 8  
December 10

Millennium Advanced Training:  
June 9 & 10  
October 27 & 28

System Suitability/GLP Training:  
April 22

Contact: Mogens Hallas  
Tel: 45 46590023  
Fax: 45 46391314

## STOCKHOLM, SWEDEN

Millennium 2010 Chromatography  
Manager Seminars:  
April 27 & 28  
September 14 & 15  
Millennium 2010 Chromatography  
Manager/996 PDA Seminars:  
April 29  
September 16

Contact: Sven-Erik Kullgren  
Tel: (08) 6286960  
Fax: (08) 6286457

## ENGLAND

### CHESTER

Millennium 2010 Chromatography  
Manager Seminars:  
March 15 - 17  
May 10 - 12  
October 18 - 20  
November 22 - 24

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Figure 5

Vial	Sample Name	Function
1	int_stand_1	Inject Standards
2	int_stand_2	Inject Standards
3	int_stand_3	Inject Standards
4	int_stand_4	Inject Standards
5	int_stand_5	Inject Standards
6	int_sam_1	Inject Samples
7	int_sam_2	Inject Samples
8	int_sam_3	Inject Samples

Figure 7

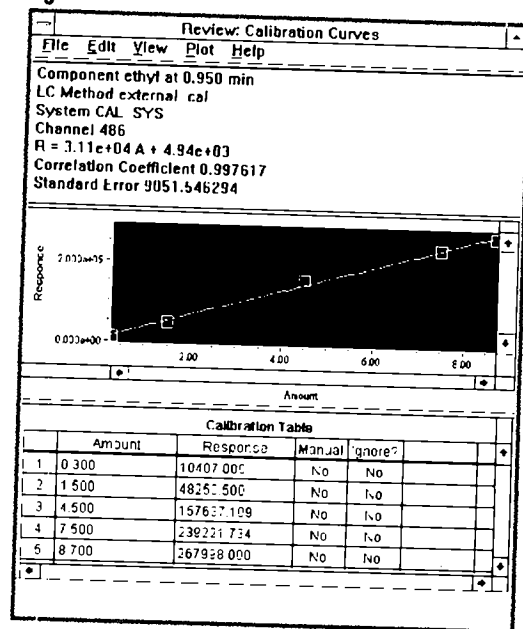


Figure 6

Vial	Sample Name	Function
1	int_stand_1	Inject Standards
2	int_stand_2	Inject Standards
3	int_stand_3	Inject Standards
4	int_stand_4	Inject Standards
5	int_stand_5	Inject Standards
6	int_sam_1	Inject Samples
7	int_sam_2	Inject Samples
8	int_sam_3	Inject Samples

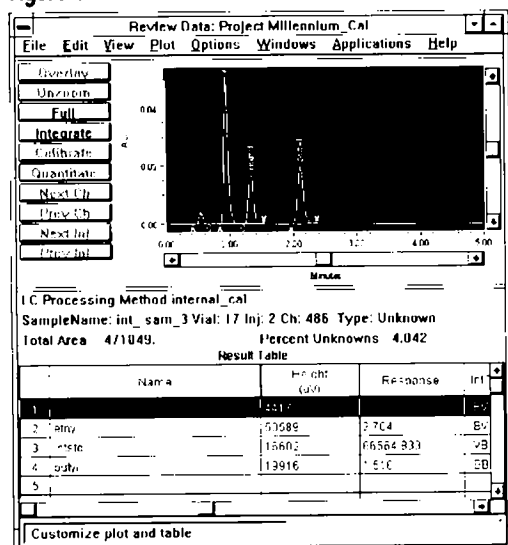
Once the standards and internal standard information is entered, you save this as part of the Method Set as described above. You then enter Quickset Control and establish the standard and sample injection sequence. As noted previously, the Component Loading Table must be filled out for each level of standard used in defining the calibration curve (Figure 5). The features Copy Components From Method and Copy Components From Previous Sample are used again to limit repetitive data entries.

The Millennium Chromatography Manager gives you the ability to select a line in the Sample Loading Table of Quickset Control and edit the Component Loading Table. Three situations involving the use of internal standards can be addressed by the Component Loading Table.

- 1) If the amount of internal standard added to the standards and samples is the same, the Millennium Chromatography Manager requires that 1.0 be entered in the Component Loading Table under the Amount column for the standards only. You are not required to edit the Component Loading Table for the samples in this situation since an amount of 1.0 is assumed.
- 2) For Good Laboratory Practices (GLP) purposes you may be required to enter the actual amount of internal standard added to the standards and samples. If so, for each line in the Sample Loading Table, the Component Loading Table needs to correctly reflect the amount of internal standard added.
- 3) If the amount of internal standard added to the sample(s) is different than that added to the calibration standards, the actual amount of internal standard must be entered for all standard and sample lines in the Sample Loading Table.

For example, in Figure 6 the amount of internal standard added to the sample was different than that of the standards and thus must be compensated for in the analysis. Figure 7 shows the multilevel calibration curve and

Figure 8



associated coefficients of the linear least squares curve fit using the method of internal standard quantitation. Notice that the Response column in Review is now the ratio of the area of the standard peak to the area of the internal standard peak. The area ratio calculated for the samples can be seen in the Results Table in Review (See Figure 8).

The Millennium Chromatography Manager has taken advantage of the Windows® Graphic User Interface (GUI) by linking the essential elements of the calibration routines in a manner that simplifies the entire process without compromising the features that define a dynamic data system. The ease of use during the calibration process coupled with the On-line Help make this system able to adapt to even the most demanding laboratory needs.

## In Brief...

**For general and specific help, don't forget the On-line Help Menus.**

- **Help Menus** - All windows in the Millennium Chromatography Manager include a Help menu. From the Help menu, you can display:
  - The main Help table of contents.
  - Help on using Help.
  - Help and step-by-step procedures for the window you are viewing.
  - Basic procedures for using the Millennium Chromatography Manager.

- **Searching for Help** - You can find help on specific topics by using the help Search feature.

Click the Search button at the top of the window and type the word you want to search for (you don't have to type the entire word - the word list jumps ahead to the letters you type), then click the Show Topics button. Select the topic to view, then click the Go To button.

# ON TARGET

We designed *on-line* with you in mind.

Now we need your input to tell us how we are doing. Was the article presented of interest to you? What topics would you like to see covered in the future? Or maybe you have an article or success story discussing applications for the Millennium Chromatography Manager.

Either way, we want to hear from you.

Call us at 508/478-2000 and ask for either Judith Rossi (x3602) or Dawn Rogers (x2436), or fax us at 508/478-5839, or call your local subsidiary.

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Andy Billingsley  
Tel: 0244-681300  
Fax: 0244-681394

## BRUSSELS, BELGIUM

Millennium 2010  
Chromatography Manager  
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Tel: (02) 2421740  
Fax: (02) 2422785

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Contact: Timo Nousiainen  
Tel: (358) 08019077  
Fax: (358) 08014640

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Call or Fax for Dates and Times  
Contact: Ronald Haas  
Tel: (06108) 22000  
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## VOLKETSUWIL, SWITZERLAND

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Contact: Paul Flueckiger  
Tel: (01) 945 3342  
Fax: (01) 945 3766

## TOKYO, JAPAN

Millennium 2010  
Chromatography Manager  
Seminars:  
April 2 & April 6  
August 3 & August 6  
October 1 & October 7  
Contact: Customer Training  
Tel: (81) 3 3471 7191  
Fax: (81) 3 3471 7116

# Questions and Answers



***I am building a Photodiode Array (PDA) spectrum library from one of my standards. The chromatogram has about twenty peaks and I have a Component Table already set up in the LC Review window. Is it necessary to retype all the component names to build the library or is there an easier way to do it?***

There is a way to cut and paste the component names between the LC Review Component Table and the Spectrum Review window. It requires having both the LC Review Component window and Spectrum Review window open and side-by-side if possible. Click on the Component Name field in LC Review then press Ctrl-C to copy the component name. Click on the Component Name field in Spectrum Review and press Ctrl-V to paste the name into the field. This should prove to be quicker than retyping names into each file, especially when long, coded component names are used. If you have only a few peaks with short names, it is probably quicker to just type in the names.

***I am trying to update my Millennium software to the new version, but my system automatically boots into the original software. When I try to install the new software (by running setup.exe on the Setup disk), I get a message telling me that there is not enough memory to run the setup program. How can I install the software in this case?***

In order to successfully install a new version of Millennium software or an existing Millennium system, it is sometimes necessary (especially in systems with 12Mb of RAM) to remove the Oracle Database program from RAM first. This can be done by exiting Microsoft Windows® and typing rempme, then pressing Enter at the DOS prompt. This will free up enough memory to run the installation program. Run Windows again by typing win /s and rerun setup.exe on the Millennium Setup disk to install the software.

***I would like to be able to switch quickly between the PDA Review, LC Review and the Project windows as well as the Microsoft Windows Program Manager. What is the best way of doing this without having to minimize one Window and maximize another.***

There are two ways of accomplishing an easy switch between applications in Windows. One way is for you press the Ctrl and Esc key simultaneously; the Windows task list will appear. Double-click on the application you wish to switch to and it will pop to the front. Another way is to hold down the Alt key then press and release the Tab key repeatedly until the name of the application you wish to switch to appears. Release the Alt key and the window will pop to the front.

***I would like to boot into Windows when I turn on the system without having the system automatically boot into the Millennium software. What do I have to change to do this?***

For ease of use and GLP reasons, Millennium systems are programmed to boot automatically into the software. This is done by having the autoexec.bat file call the c:\milenium\script\milenium.bat file. The last line of the milenium.bat file contains the command win /s milenium.exe. This instructs DOS to load Microsoft Windows and run the milenium.exe application. If you change the last line in the milenium.bat file to read win /s, the batch file will load Oracle and all other necessary commands for the Millennium software but will only boot into Windows. You can then run Millennium software by double-clicking on the Session Manager icon in the Millennium Program Group.

NOTE: The spelling of "milenium" is different when it appears as a DOS file or directory name. The DOS file name is shortened due to the eight character DOS file limitation.

# What's New?

At Pittcon, Millipore will be introducing:

## The Millennium™ 2020C/S Chromatography Networks

Millennium 2020C/S networks are the first laboratory networks to offer true client/server architecture with central database management. Designed for labs that require networking and centralized data management, the Millennium 2020C/S will provide integrated information management for the entire chromatography lab with the same software functionality as the Millennium 2010 Chromatography Manager.

Networks will be available for shipment in the second half of 1993 for Novell NetWare™ and VMS/PathWorks™. Here's what you can expect:

- Real-Time Acquisition — Access to any chromatograph on the network from any PC on the network.
- Central Data Management — Central sharing, tracking, trending, and management of raw data, methods, and results for all chromatographs on the network.
- GLP Benefits — Enhanced data, methods, and results security from accidental overwrite, deletion or tampering.

If you would like more information, just return the enclosed reply card.

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## Millennium 2010 Chromatography Manager V1.1 — Released!

**In December and January, we shipped Version 1.1 of the Millennium 2010 Chromatography Manager to all new and existing customers. This new release adds several major enhancements requested by our customers:**

- ASCII export of raw data and results to other applications.
- Operating procedures for additional calibration capabilities and enhanced ease-of-use.
- Multi-system and SIM support.
- System Suitability/Methods Validation Option including a variety of tracking and trending capabilities.
- Interactive pump control, allowing fast, easy changes to solvent conditions.
- Millennium GPC Manager, including Reference Peak and advanced calibration routines.
- Upgrades for Waters 991 photodiode array detectors to Millennium compatibility.

Contact your sales representative for additional information on any of these exciting new features!



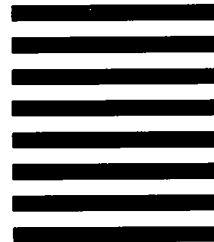
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with the latest version of the Millennium Chromatography Manager including GPC, System Suitability and now networking capabilities.

### Please send me information on the following Millennium Products:

- ☐ 3 Millennium 2020C/S Networks
- ☐ 4 Millennium Gel Permeation Chromatography
- ☐ 5 System Suitability Option
- ☐ 6 996 Photodiode Array Detector

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