A simple method for transferring Shimadzu C-R6A Chromatopac data to a Macintosh based graphics package via an IBM PC: application to the HPLC analysis of paralytic shellfish poisoning toxins

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A Simple Method for Transferring Shimadzu C-R6A Chromatopac Data to a Macintosh® Based Graphics Package *via* an IBM PC. Application to the HPLC Analysis of Paralytic Shellfish Poisoning Toxins.*

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Abstract

With particular application to the post-column reaction/fluorescence detection (PCR/FD) high performance liquid chromatographic (HPLC) analysis method for paralytic shellfish poisoning (PSP) toxins, a semi-automated procedure for converting Shimadzu C-R6A Chromatopac data to a Macintosh based graphics package (MacDraw®) *via* an IBM PC was developed. The method eliminated the need to physically *cut and paste* chromatograms into Microsoft® Word documents and, for ease of comparison of multiple chromatograms, it allowed for the generation of stacked chromatographic plots.

Introduction

As part of an ongoing program to develop instrument calibration solutions and reference materials for paralytic shellfish poisoning (PSP) toxins, an in-house post-column reaction/fluorescence detection (PCR/FD) high performance liquid chromatography (HPLC) system[1] was set up and extensively tested.[2] In order to electronically archive the HPLC data, a Shimadzu C-R6A Chromatopac integrator, used to record and integrate the PSP toxin HPLC traces, was interfaced to an IBM PC using an RS-232 communications board and the Shimadzu Archive Data Utility software package. The Chromatopac to IBM PC linkup also facilitated replotting of the electronically archived chromatograms on the Chromatopac. In order to include the archived chromatograms in technical reports[2] and publications,[3] it was necessary to replot the data on the Chromatopac and to *cut and paste* the chromatograms into documents. Although this was not a problem for single chromatographic traces, it was impossible, using the *cut and paste* method, to generate stacked plots of chromatograms that would graphically illustrate the differences between individual sample analyses.

Many companies offer commercial chromatographic interfaces, most of which include sophisticated graphics programs. In general, however, these interfaces are prohibitively expensive and they would make the Shimadzu C-R6A Chromatopac currently in use, a $5,500 investment,

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[1]Sullivan, J.J.and M.M. Wekell. 1987. The application of high performance liquid chromatography in a paralytic shellfish poisoning monitoring program. In Kramer, D.E. and J. Liston (eds.) *Seafood Quality Determination,* Proceedings of the International Symposium on Seafood Quality Determination. New York, NY: Elsevier Science Publishing, pp 357-371.

[2]Ayer, S.W., W.G. Styles, M.N. Quigley, R. Guevremont, P.G. Sim, and J.L.C. Wright. 1990. Pre-collaborative study of the high performance liquid chromatographic method for paralytic shellfish poisons. Atlantic Research Laboratory Technical Report No. 60, pp 1-91.

[3]Quilliam, M.A., S.W. Ayer, S. Pleasance, P.G. Sim, and P. Thibault. Recent developments in instrumental analytical methods for marine toxins. Seafood 2000 Proceedings, *in press.*

obsolete. As our goal was to be able to include stacked chromatographic traces in Macintosh based Microsoft Word documents, we chose to transfer the IBM PC based chromatographic data to a Macintosh SE personal computer using software that already existed in our laboratory.

Experimental

Hardware: Shimadzu C-R6A Chromatopac recording integrator equipped with an RS-232 communications board. Macintosh SE personal computer with a 40 Mb internal hard disk drive. Compatible 286 with a 40 Mb internal hard disk drive.

Compatible 286 Software: Microsoft DOS 4.01; Chromatopac Data Archive Utility Version 1.0.

Macintosh SE Software: System Software 6.0.4; Apple® File Exchange System Software 1.1.3; Cricket Graph™ 1.3.1; Microsoft Excel 2.2a; Microsoft Word 4.0; MacDraw 1.9.5.

Manual procedure for converting one chromatographic data set.

1. Load the Microsoft Excel program.
2. Open the required IBM/Shimadzu file as a comma separated text file (IBM/Shimadzu files end with the file designation .PRN).
3. Insert a blank column between the the two columns containing the time and detector output values.
4. Cut the time values in column 1 from row 1,801 to row 3,600 and paste in column 2 starting in row 1. Repeat for the same rows in column 3, pasting the cut values into column 4.
5. Save the file as a text formatted Microsoft Excel file.
6. Quit Microsoft Excel and load the Cricket Graph Program.
7. Open the Microsoft Excel file saved in step 5.
8. Check the Cricket Graph column format to make sure that it is in the decimal form.
9. The data is now ready to be used for plotting or to be saved as a Cricket Graph graph file.

Automated procedure for converting more than one chromatographic data set.

1. Make a backup copy of the *filename*.PRN designated chromatographic data.
2. Load the Microsoft Excel program.
3. Open the Microsoft Excel *macro* entitled *Macro IBM to Cricket.*
4. Modify line 8 of the *macro* to correspond to the number of files to be converted (e.g. "Count",1,7,1 would be changed to "Count",1,4,1 for conversion of 4 files).
5. After selecting row number one, run the *macro.*
6. When the file conversion is complete, quit Microsoft Excel and load Cricket Graph.
7. Open the file produced in step 5.
8. Copy the file names from row 1 into the Cricket Graph column headings.
9. Change all the columns from text to decimal format.
10. The data is now ready to be used for plotting or to be saved as a Cricket Graph graph file.

Procedure for transferring a Cricket Graph graph file to MacDraw.

1. To import a Cricket Graph drawing in MacDraw, save the Cricket Graph graph as a PICT formatted file.
2. Load MacDraw.
3. Open the Cricket Graph file saved in step 1.
4. Cut parts of the graph, as required, using MacDraw.
5. In order to generate stacked plots, *cut and paste* multiple chromatograms using MacDraw.

Results and Discussion

The IBM PC based Shimadzu Data Archive Utility package was able to transform the data from the electronically archived chromatographic traces into either ASCII or DIF files. Using the ASCII file format, the IBM PC data was transferred using the Apple File Exchange program based on the Macintosh SE. The chromatographic data was in a two column comma separated value (CSV) format as shown in Table 1.

Next we investigated the possibility of importing the chromatographic data directly into Cricket Graph, a powerful Macintosh based graphics package. Unfortunately, Cricket Graph required tab separated values (TSV's) and was limited to 2,700 rows of time and detector output

Table 1. Two column CSV data format generated by the Shimadzu Data Archive Utility Program.[4]

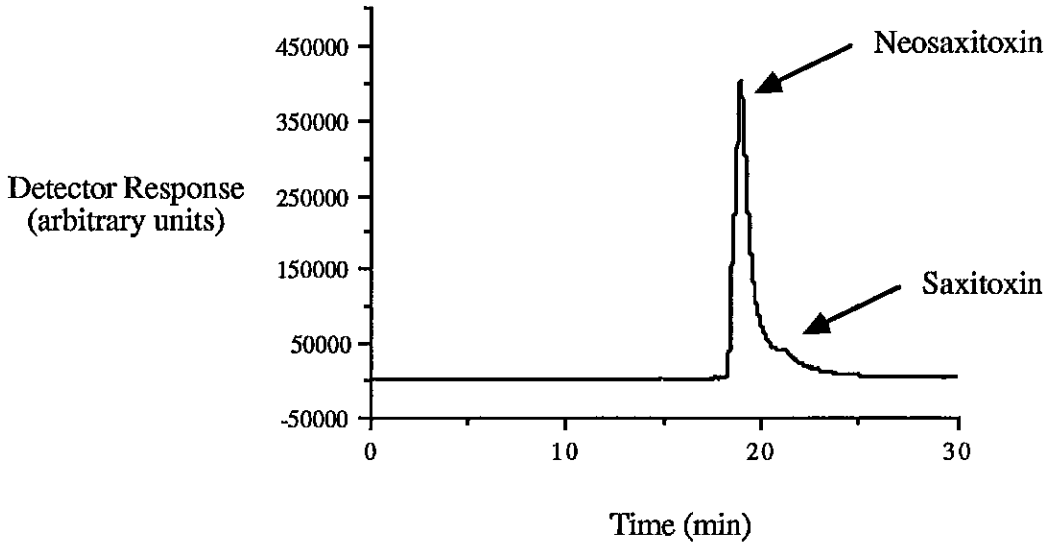
0.000,751
0.008,737
0.017,689
0.025,753
0.033,610
0.042,770
0.050,822
0.058,893
0.067,829
0.075,731
0.083,916
0.092,817
0.100,594
0.108,634
0.117,587
etc.

values. The standard thirty minute PSP toxin HPLC analysis produced 3,600 rows of data. In order to overcome these Cricket Graph limitations, we found that the Macintosh based Microsoft Excel package could read CSV's into two separate columns and it would readily accept 3,600 rows of CSV data. Unfortunately, the graphics available with the Microsoft Excel package were inadequate when compared to Cricket Graph. In order to use Cricket Graph for plotting, we were successful in splitting the two column CSV data set of 3,600 rows (Table 1) into four columns of 1,800 rows per column[5] and saving the data as a TSV text file in Microsoft Excel. The resulting TSV Microsoft Excel text file was readable by Cricket Graph. An example of a Cricket Graph plot of a PSP toxin HPLC chromatogram produced in this way is shown in Figure 1. It is important to point out that, when generating the graph shown in Figure 1, it was necessary to plot the first 1,800 time and detector output values, and then to overlay the second 1,800 time and detector output values on top of the first using the overlay feature of the Cricket Graph package.

Due to the fact that an operator is needed to initiate each step of the data conversion when more than one chromatogram is to be plotted, as is the case for stacked plots, the method described above was very time consuming. Therefore, we developed a procedure for the automated CSV to TSV conversion of multiple chromatograms. A Microsoft Excel *macro* was written to read multiple two column CSV files, split each file into four parts as described above, and export the

[4]The first value in each row represents time, in minutes, while the second value is the digital representation of the fluorescence detector output at the time specified by the first value.
[5]Two time and two detector output columns.

Figure 1. Cricket Graph plot obtained from the PCR/FD analysis of a fraction rich in neosaxitoxin.



data into a Cricket Graph readable file. The Microsoft Excel *macro* is outlined in Table 2. Table 3 shows a portion of the Microsoft Excel file that resulted from reading multiple two column CSV chromatographic data sets. Notice that for standard thirty minute PSP toxin chromatograms, it was only necessary to enter the time values in the first two columns of a multiple chromatographic data set. The file names for the detector output values were automatically entered above the first column of each two column detector output data set. Table 4 shows the Cricket Graph file containing the same data set after importation from Microsoft Excel.

There were two types of files which both Cricket Graph could read and Microsoft Excel could write. These were a tab separated text file and a SYLK file (a SYLK file is a file output format devised and used by Microsoft in its products). For seven thirty minute chromatograms containing sixteen 1,800 row columns (recall that the time values only required two columns), a tab separated file required 149K of memory and required 235[6] seconds to be read by Cricket Graph. In contrast, a SYLK file required 340K and took 310 seconds to be read. Because they were the fastest and the least memory demanding, it was decided to transfer Microsoft Excel data as a tab separated text file.

Once the data was properly formatted, Cricket Graph could save data in two ways, as a text or a graph file. A Cricket Graph text file containing the data for seven thirty minute PSP toxin chromatograms required 315 seconds to write the file to the hard disk and 410 seconds to read the

[6]All times refer to processing on a Macintosh SE personal computer. Processing on a Macintosh II is significantly faster.

Table 2. Microsoft Excel *macro* for converting more than one chromatographic data set.

1	IBM to Cricket graph format
2	=DIRECTORY("Ayer:Applications:Cricket Graph Folder:Shimadzu/IBM Data:Chromatograph Data")
3	=DIRECTORY("Ayer:Applications:Cricket Graph Folder:Shimadzu/IBM Data")
4	=OPEN("Time Worksheet",,,1)
5	=ACTIVATE("Time Worksheet")
6	=SELECT("R1C2")
7	=DIRECTORY("Ayer:Applications:Cricket Graph Folder:Shimadzu/IBM Data:Chromatograph Data")
8	=FOR("Count",1,7,1)
9	=FILES()
10	=MESSAGE(TRUE,A9)
11	=OPEN(A9,,,2)
12	=ACTIVATE("Time Worksheet")
13	=SELECT("R1C[1]")
14	=SET.NAME(A9,1)
15	=DEFINE.NAME(A9,A9,1,)
16	=FORMULA(LEFT(A9,8))
17	=ACTIVATE(A9)
18	=SELECT("R1C2:R1800C2")
19	=COPY()
20	=ACTIVATE("Time Worksheet")
21	=SELECT("R[1]C")
22	=PASTE()
23	=ACTIVATE(A9)
24	=SELECT("R1801C2:R3600C2")
25	=COPY()
26	=ACTIVATE("Time Worksheet")
27	=SELECT("R2C[1]")
28	=PASTE()
29	=SELECT("R1801C")
30	=COPY()
31	=ACTIVATE(A9)
32	=CLOSE(FALSE)
33	=BEEP()
34	=FILE.DELETE(A9)
35	=NEXT()
36	=DIRECTORY("Ayer:Applications:Cricket Graph Folder:Shimadzu/IBM Data")
37	=SAVE.AS(LEFT(A9,4),2,FALSE)
38	=RETURN()

entire data file back from the hard drive. Data saved as a Cricket Graph graph file required only 80 seconds to read or write the entire file.

Table 3. Portion of Microsoft Excel file that resulted from reading multiple two column CSV chromatographic data sets.

	A	B	C	D	E	F	G	H
1	time	time	AG153110		AG153312		AG153517	
2	0	15	516	30	-169	-135	1451	1388
3	0.008	15.008	703	-253	29	-266	1820	1747
4	0.017	15.017	1003	-283	-354	-224	1646	1551
5	0.025	15.025	628	-230	-154	101	1424	1994
6	0.033	15.033	744	-334	-16	118	1692	1707
7	0.042	15.042	717	-433	-153	-61	1549	1798
8	0.05	15.05	643	-366	-156	-316	1293	1781
9	0.058	15.058	861	-361	-558	-321	1450	1868
10	0.067	15.067	909	-271	-51	-44	1845	1597
11	0.075	15.075	531	-408	-31	-5	1497	1717
12	0.083	15.083	515	-601	141	131	1741	1666
13	0.092	15.092	887	-298	-48	31	1574	1777
14	0.1	15.1	1045	-411	-130	144	1468	1846
15	0.108	15.108	907	-59	83	241	1630	2054
16	0.117	15.117	839	-144	377	-15	1601	2059
17	0.125	15.125	718	182	215	-189	1363	1551
18	0.133	15.133	684	-175	304	-44	1251	1663
19	0.142	15.142	732	-217	258	-217	1294	1553
20	0.15	15.15	631	-349	162	-296	1371	1613
21	0.158	15.158	977	-243	-304	25	1657	1715

In order to generate stacked plots of chromatograms (see Figure 2), the individual chromatograms were generated using Cricket Graph and the Cricket Graph graph files were saved as PICT formatted files.[7] The PICT files were then loaded in MacDraw and standard MacDraw techniques were then used to manipulate the chromatograms to generate stacked plots.

Conclusion

A cost effective method for transferring IBM PC based chromatographic data directly into Macintosh based Microsoft Word documents was developed. The ability to generate stacked plots of PSP toxin PCR/FD HPLC chromatograms, and to incorporate the stacked plots directly into Microsoft Word documents, greatly enhanced the visual presentation of the chromatographic data. The method could readily be applied to a broad range of IBM PC to Macintosh data manipulation processes.

[7]It should be noted that PICT formatted files, while readable by MacDraw, are not readable by Cricket Graph.

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Table 4. Cricket Graph data file containing the same Microsoft Excel chromatographic data set (Table 3) after importation into Cricket Graph.

	1	2	3	4	5	6	7	8
	time	time#2	AG153110	AG153110#2	AG153312	AG153312#2	AG153517	AG153517#2
1								
2	0.000	15.000	516	30	-169	-135	1451	1388
3	0.008	15.008	703	-253	29	-266	1820	1747
4	0.017	15.017	1003	-283	-354	-224	1646	1551
5	0.025	15.025	628	-230	-154	101	1424	1994
6	0.033	15.033	744	-334	-16	118	1692	1707
7	0.042	15.042	717	-433	-153	-61	1549	1798
8	0.050	15.050	643	-366	-156	-316	1293	1781
9	0.058	15.058	861	-361	-558	-321	1450	1868
10	0.067	15.067	909	-271	-51	-44	1845	1597
11	0.075	15.075	531	-408	-31	-5	1497	1717
12	0.083	15.083	515	-601	141	131	1741	1666
13	0.092	15.092	887	-298	-48	31	1574	1777
14	0.100	15.100	1045	-411	-130	144	1468	1846
15	0.108	15.108	907	-59	83	241	1630	2054
16	0.117	15.117	839	-144	377	-15	1601	2059
17	0.125	15.125	718	182	215	-189	1363	1551
18	0.133	15.133	684	-175	304	-44	1251	1663
19	0.142	15.142	732	-217	258	-217	1294	1553
20	0.150	15.150	631	-349	162	-296	1371	1613
21	0.158	15.158	977	-243	-304	25	1657	1715

Figure 2. Stacked plot of chromatograms generated using MacDraw. A7 and A4 were saxitoxin standards at 1 and 6 μg/mL, respectively. The other analyses were of fractions obtained from a Bio-Rex 70 purification of neosaxitoxin.