PA13/30 HPLC'99 Granada, 31 May 1999

# From Data to Document:

# A Simple Way to Prepare High-Quality Chromatograms for Electronic & Paper Publication

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# Abstract



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Many opportunities are available today for chromatographers to publish their experimental results -- articles in refereed journals, internal lab reports, internet web pages, etc. How often, though, do you see chromatograms in these publications with jagged or skewed peaks, inconsistent line thickness, inappropriate scales, or a hand-drawn appearance?

One of the stumbling blocks in getting high-quality illustrations into electronic or paper documents is dealing with the data from a chromatographic system. Over the years, many "homemade" solutions have been devised to meet an immediate goal: paste a strip chart onto a poster panel, photocopy a portion of a data system printout onto a report page, place a WMF or HGL file into a word processor or presentation program document, etc.

However, creating a publication-ready chromatogram which can be moved from the PC in the lab to the Mac at the publishing house, transferred directly from one software application to another, scaled up or down in size, or reformatted as needed -- all the while maintaining the resolution and appearance of the original -- has become tedious and complicated for those who are not skilled in technical graphics design and computer graphics formats.

A simple protocol has been developed, using off-the-shelf software, to take raw data (as ASCII text) from a data system and create a high-quality chromatogram in a cross-platform, scalable, standard file format. This graphics file may be manipulated in high-end illustration programs, placed in simple documents and presentation files, published on the web in several ways, and/or archived for future use. This protocol is not limited to HPLC, but, in principle, may also be applied to CE, CEC, GC or MS data.

Limitations of previous solutions will be outlined. Applications and advantages of the new protocol will be illustrated.

# Dead End Chromatograms



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## Before PCs:

- Paste directly into paper or notebook:
  - Actual strip chart, plotter, printer output
  - Photograph, photocopy, hand–drawn ink
- Send original or glossy photo to printer for reproduction

## After PCs:

- From data system: print to paper
- Copy/Paste HGL, wmf, pict into electronic presentation [Freelance*, Powerpoint*, etc.]

*Freelance is a trademark of Lotus/IBM Corporation; Powerpoint is a trademark of Microsoft Corp.

# Typical Illustration Problems



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This chart was meant to illustrate batch-to-batch reproducibility via consistent peak retention times.

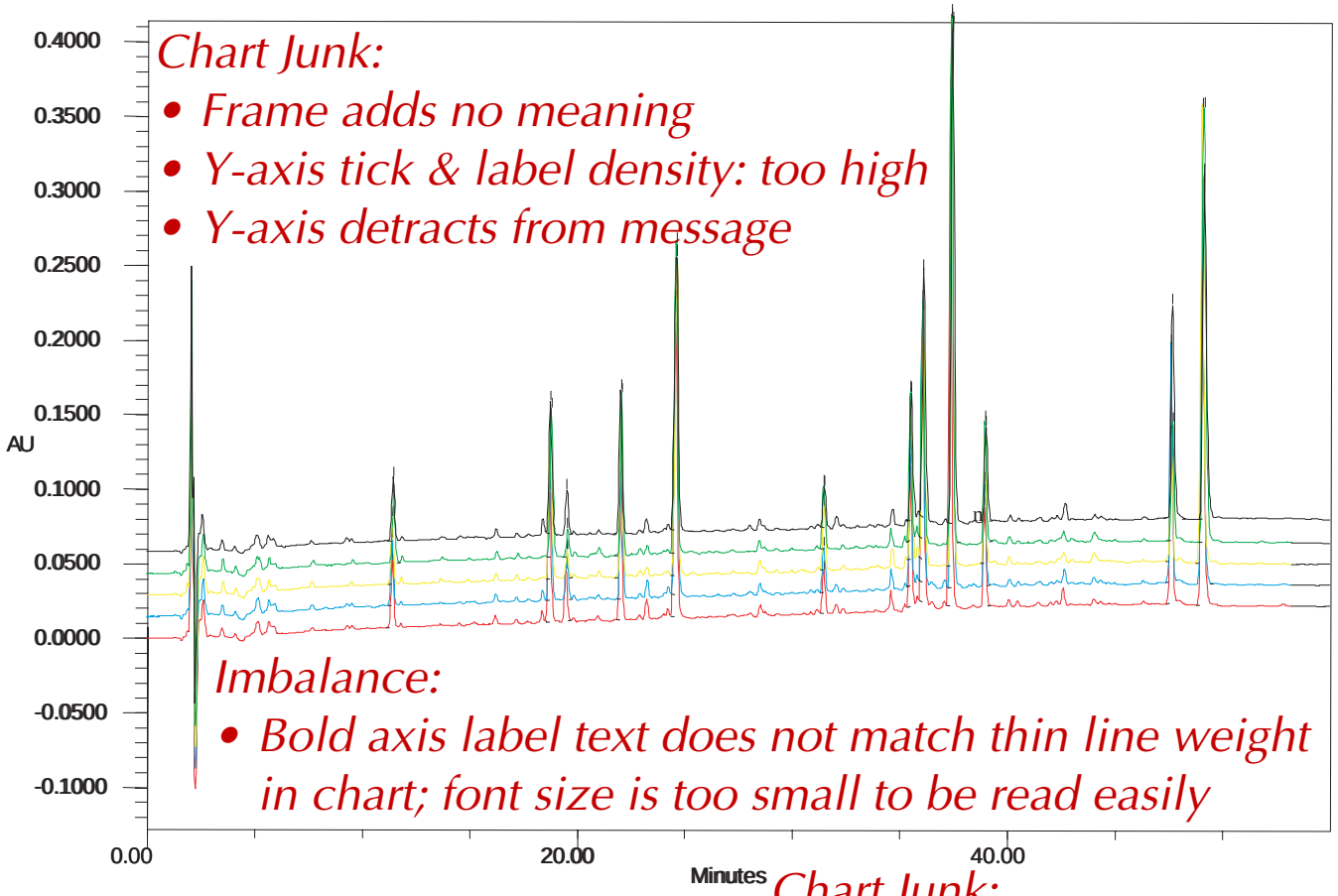


*Chart Junk:*

- *Frame adds no meaning*
- *Y-axis tick & label density: too high*
- *Y-axis detracts from message*

*Imbalance:*

- *Bold axis label text does not match thin line weight in chart; font size is too small to be read easily*

- *Chromatograms are too close to each other for easy visual comparison*
- *Peaks are skewed & "stepped"*
- *Chromatograms do not appear smooth if scaled up or down in size*
- *HGL chromatograms "placed" into Freelance — Illustration is not "exportable"*

*Chart Junk:*

- *Legend takes attention away from data & reduces space available for chart*
- *Pale colors are hard to discern in thin lines*
- *Text labels are coded & confusing*

# Ideal Chromatograms



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- Capture, export & store data *electronically* in secure, networked archive
- Retrieve data with any client platform, *independent* of original capture system
- Create custom electronic chromatogram in graphics format *portable* across:
  - Software packages [graphics, drawing, presentation, document creation, etc.]
  - Operating systems: [WIN, Mac OS, etc.]

# Graphics Formats



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## *Three Types of Interchange Formats:*

- Bitmap files: *a matrix of square pixels*
  - e.g., TIFF* (tag image file format); BMP (Windows bitmap); MacPaint; PCX (PC Paintbrush)
- Vector files: *math instructions for drawing objects*
  - e.g., Autocad; DXF (dynamic exchange format); Postscript paths & type*; HGL (H-P plotter graphics language)
- Metafiles: *contain vector and/or bitmap graphics*
  - e.g., PICT (Mac); WMF (Windows metafile); Adobe Illustrator EPS* (encapsulated postscript); CGM (computer graphics metafile)

* ***Device independent:*** designed for interchange between different computing environments

# Recommended Formats*



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| ***If file format is:*** | ***To publish it:*** | ***Convert it to:*** *[file suffix]* |
|---|---|---|
| DXF, vector metafiles, or metafiles with both bitmap & vector elements | On paper via Postscript | EPS [.eps] |
| PCX, BMP, or bitmap metafiles | On paper via Postscript | TIFF [.tif] |
| DXF or vector metafiles | Online | GIF [.gif] |
| PCX, BMP, TIFF, or bitmap metafiles | Online | GIF or JPEG [.gif or .jpg] |

* O.M. Kvern, "*Desktop Science: The Right Match. Don't get burned by the wrong graphics file format,*" Adobe Magazine, Spring 1997, **8(2)**, 57-60; I highly recommend reading this short article which is available as a pdf file: <http://www.adobe.com/publications/adobemag/archive/PDFS/9704dsok.pdf>

# Creating an EPS Chromatogram



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- Step 1: *collect* raw data; *export* to text file
  - TOOL: Waters Millennium®32 Software v. 3+ (PC)*

  *Advantages:* Can export x,y coordinates with selected delimiters
- Step 2: *plot* data; *export* chart as EPS [or JPEG]
  - TOOL: SPSS Deltagraph, v.4.5 (Mac) or v. 4.05 (PC)**

  *Advantages:* Plots ~40,000 data points & exports desired formats; makes templates for fast chart creation with auto-formatting
- Optional: *customize* chart; *save* as EPS
  - TOOL: Adobe Illustrator, v. 8 (Mac or PC)***

  *Advantages:* Can parse EPS file & selectively modify any element

* <http://www.waters.com/Waters_Website/Millennium32/M32top.htm>;
** <http://www.spss.com/software/deltagraph>; *** <http://www.adobe.com/prodindex/illustrator/main.html>

# Alternative Software Limitations



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- *Data Export:* Earlier versions of Millennium® Software could only set delimiter for x–value; word processor was then used to convert default *hard space* after each y–value in text file to a *return*.

  We have not tested ability of other data acquisition software to *export* raw *x,y data* as a comma- or tab-delimited *text* file.

- *Plotting:* Most programs examined have one or both limitations:
  - they cannot *export* a formatted EPS or JPEG chart [e.g., Excel*]
  - they cannot *plot* a large number of data points [$>10^4$].

- *Modification:* We have not tested tools outside the Adobe family for their capability to *parse* & *modify* an EPS graphic.

* Excel is a trademark of Microsoft Corporation

# Step 1 – Export Raw ASCII Data



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- Set up export method [once] for raw data in Millennium32
- Choose delimiters:
  after x value: *comma* [,]
  after y value: *return* [↵]
- Export only x,y data (no headers, etc.) to a text file
- Change [rename] suffix:
  *.*arw* ➔ *.*txt*
- Viewed in a word processor, an unformatted text file appears as at right [*a long list of x,y values – one per line*; File Size: only 208K]

8.333333e-03,1.150000e-04
1.666667e-02,1.190000e-04
2.500000e-02,1.170000e-04
3.333333e-02,1.090000e-04
4.166667e-02,9.900000e-05
5.000000e-02,9.400000e-05
5.833333e-02,9.700000e-05
6.666667e-02,1.070000e-04
7.500000e-02,1.190000e-04
8.333333e-02,1.270000e-04
9.166667e-02,1.270000e-04
1.000000e-01,1.210000e-04

7800 x,y values

6.490000e+01,-2.700000e-05
6.490833e+01,-1.900000e-05
6.491667e+01,-1.500000e-05
6.492500e+01,-1.700000e-05
6.493333e+01,-2.500000e-05
6.494167e+01,-3.500000e-05
6.495000e+01,-4.400000e-05
6.495833e+01,-4.700000e-05
6.496667e+01,-4.100000e-05
6.497500e+01,-3.200000e-05
6.498333e+01,-2.300000e-05
6.499167e+01,-1.900000e-05
6.500000e+01,-2.300000e-05

# Step 2 – Import Data & Plot



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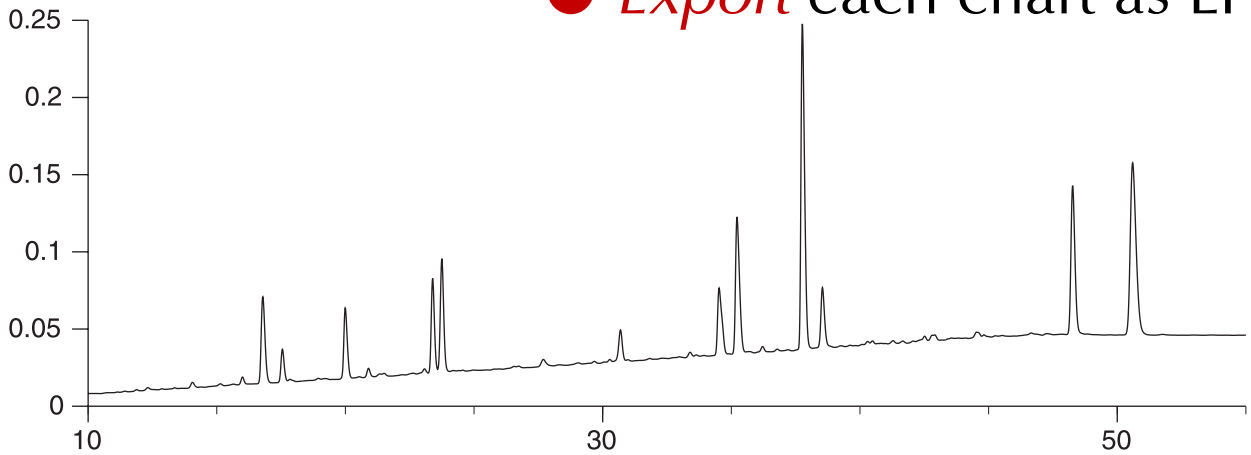
- *Import* comma-delimited text file into Deltagraph
- *Select* values from 10 to 55 min in Data view
- *Choose* x,y line in Chart Gallery & *Plot*
- *Change* parameters
  - *Chart axes:*
    ***x***: length = 6″; left value – 10, right value – 55; major increment – 20; minor division – 4
    ***y***: length = 2″*; major – 0.05; minor – 1
  - *Chart Options:* no legend; connect points;
  - *Draw/Object attributes:* line fore color – black; line weight – very thin line;
    Font – Futura Book PS; Text size – 14
- *Save* chart as template
  - use to plot additional chromatograms
- *Export* each chart as EPS file




* For multiple overlay chart; otherwise set y = 4″ for single chart ideal height:width proportion of 1:1.5

# Create Overlay Chart



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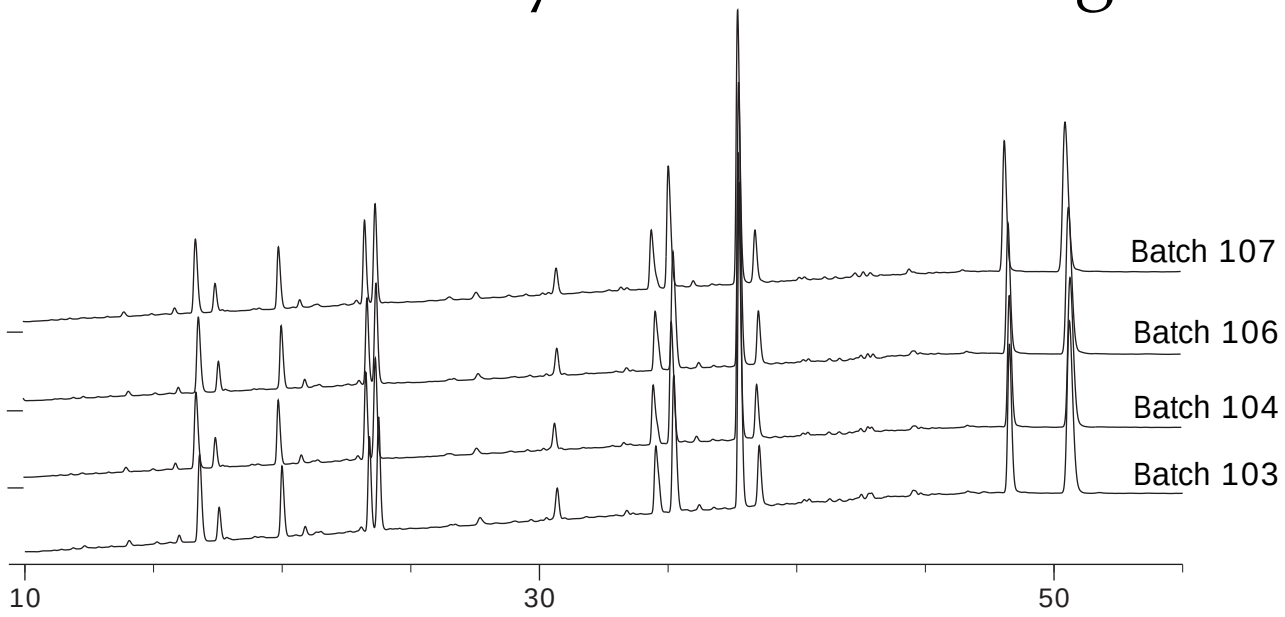
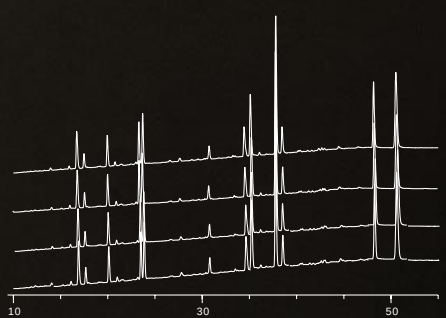
- *Open* each file as parsed EPS in Illustrator
- *Remove* unnecessary elements & resave as EPS
- *Open* EPS file containing baseline
- *Place* each subsequent EPS file, offset, into first
- *Remove* remaining elements used to align overlay
- *Annotate* as necessary & save final figure as EPS



# You're working on a drug that could save lives.

## Your company is counting on you for its success.

## And you're only 300 angstroms away.




*The most rigorous in the HPLC column industry, our batch release protocol includes a tryptic map of cytochrome c used to test for reproducibility of retention time and resolution. Here, the tryptic map was run on three Symmetry300™ columns, each containing a different batch of packing.*

Column-to-column variability, the "Achilles heel" for scientists developing HPLC assays for well characterized biopharmaceuticals. Until now, you've had to accept variability because that's what the industry offered you. You found ways to work around it, but they cost you time, money and potentially much, much more. That's why Waters has developed Symmetry300™ columns, the new standard for the next generation of biopharmaceutical HPLC assays. This new wide-pore reversed-phase column is built on the extraordinary Symmetry® column platform, the standard the pharmaceutical industry has come to rely on for sensitive, rugged and robust HPLC. Now, whether you're developing HPLC assays for purity, stability or identity, you can have confidence in the long-term compliance of your methods. We prove it by including with every column a four-page certificate of analysis that reports on Symmetry300™'s manufacturing specifications and the actual results of the 28 critical quality tests we perform on the raw silica, bonded silica and finished packed column. Set a new standard for all your HPLC assays. The Symmetry300™ standard. Column-after-column, year-after-year, no matter who uses your methods or where they're used around the world. It's the better solution you need for validation compliance. In the U.S., call us at 1-800-252-4752.In Europe FAX +33(0) 1 30 48 73 88.

## SYMMETRY® COLUMNS

Waters

Visit the Waters Web site at www.waters.com

# Example 2



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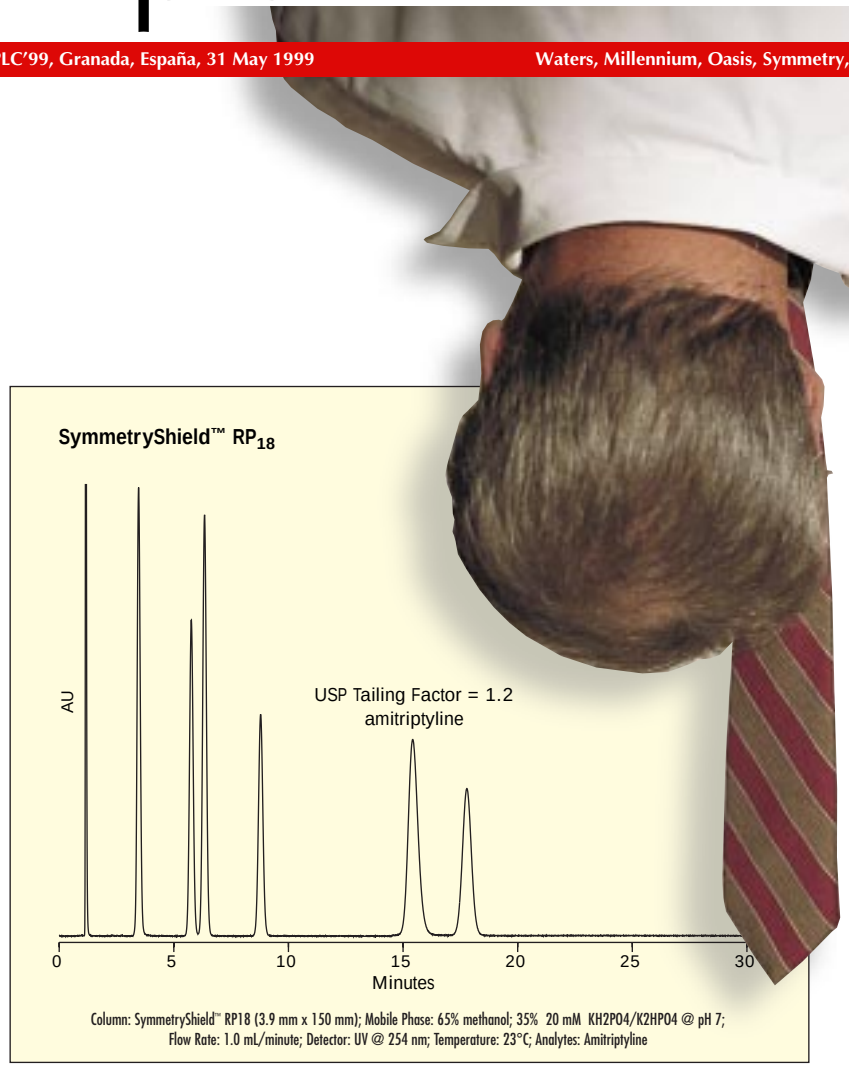


**SymmetryShield.™**

**It's a radical new way to look at HPLC columns.**

From raw data export out of Millennium[32] to chart creation in Deltagraph takes less than 5 minutes!

Designer's tools:

*Graphics:* Adobe Illustrator & Photoshop

*Page Layout:* QuarkXPress

*OS:* Mac OS.

Chromatography: Bonnie Alden

Ad Design: Bill Cloutman

# Example 3



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## Xtreme Speed and Resolution

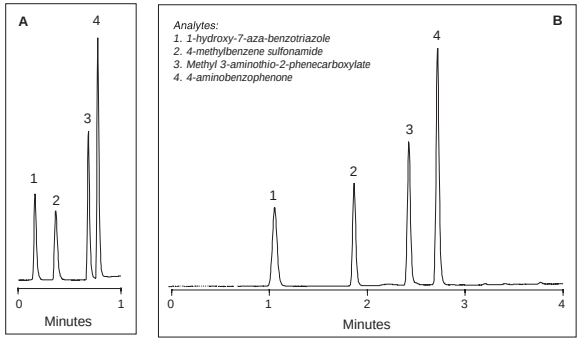
The inability to maintain efficiency at higher linear velocities with usable column backpressure puts limits on the analysis speed in your lab.

Now XTerra™ columns allow you to:

- Work at elevated temperatures, up to 80 °C —reducing system backpressure and improving column efficiency.
- Work with particles as small as 2.5 µm—maintaining maximum efficiency and high throughput.

The result is significantly reduced gradient run times with maximum resolution.

### A Three-Fold Increase in Throughput for Generic Gradients Through the Use of Short Narrowbore 2.5 µm XTerra™ Columns




| | A | B |
|---|---|---|
| Column: | XTerra™ MS $C_{18}$, 2.5 µm 2.1 x 20 mm | XTerra™ MS $C_{18}$, 5 µm 4.6 x 50 mm |
| Mobile Phase: | A: = 0.1% TFA in water<br>B: = 0.08% TFA in acetonitrile | A: = 0.1% TFA in water<br>B: = 0.08% TFA in acetonitrile |
| Gradient: | 5 – 95% B | 5 – 95% B |
| Gradient Time: | 45 seconds | 4 minutes |
| Flow Rate: | 1.6 mL/minute | 1.5 mL/minute |
| Column Temperature: | 60 °C | 30 °C |
| Detection: | UV – 254 nm | UV – 254 nm |
| Injection Volume: | 1 µL | 3 µL |

## Xtreme Peak Shape

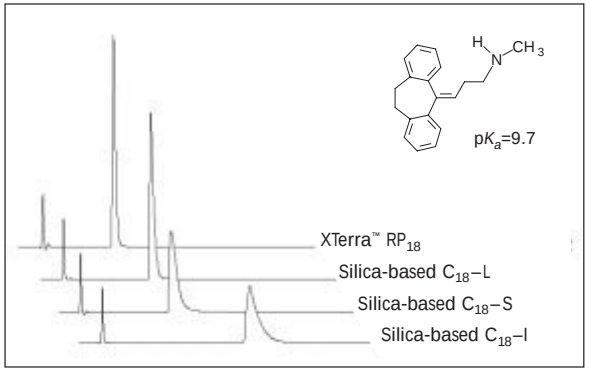
The inability to adequately reduce the number of silanols on a silica surface by more than 50% despite extensive bonding procedures puts limits on the peak symmetry in your method. Particularly for strongly basic compounds making it more difficult for you to work with generic methods.

Because hybrid particles are partially organic (they have methylsiloxane units in place of one third of the $SiO_2$ units), they yield bonded phases with increased surface coverage reducing concentrations of residual silanols. As such, XTerra™ columns deliver a significant reduction in peak tailing for basic compounds.

Now XTerra™ columns allow you to:

- Develop more reliable generic methods by maximizing peak capacity.
- Increase the sensitivity of methods

### Comparison of Nortriptyline Peak Shapes on XTerra™ $RP_{18}$ and Benchmark Columns




*XTerra™ columns show improved peak symmetry and efficiency for the strong base nortriptyline.*

| | |
|---|---|
| Mobile Phase: | 20mM $K_2HPO_4$ pH 7/acetonitrile, 65/35 |
| Flow Rate: | 1.4 mL/minute |
| Column Temperature: | 25 °C |
| Detector: | UV–254 nm |

Compare superior *quality* from our new **EPS** procedure [top left] with "stepped" peaks produced by old **HGL** methods [bottom left].

Designer's tools:

*Graphics:* Adobe Illustrator & Photoshop

*Page Layout:* QuarkXPress

*OS:* Mac OS.

Chromatography: Dr. Judy Carmody
Jeff Grassi

Deltagraph Plots: Dr. Ray Crowley

Brochure Design: Keith Finch
[Lowe Grob agency]

# Discovery: Postscript Advantage



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As a significant outcome of both our work on the foregoing procedure & with PDF creation tools (see adjacent Poster PA13/31), *we discovered an extraordinarily simple & elegant way to rescue old chromatograms* & other illustrations, for which the data files were not readily available, but which once had been placed into *still printable* electronic documents [Word, Freelance, Powerpoint, etc.].

Tools:

- Software used to create document
- Adobe Acrobat Distiller*
- Adobe Acrobat Exchange*
- Adobe Illustrator*

Procedure :

- Using original application, *print* document to ***Postscript**** file [PS]
- *Distill* **PS** to *Portable Document Format* [PDF] with Distiller
- *Export* **PDF** page to **EPS** using Exchange
- *Open* parsed **EPS** file in Illustrator; remove unwanted elements, refine illustration, save as **EPS** graphic

* Adobe, Acrobat, Distiller, Exchange, Illustrator, Postscript are trademarks of Adobe Corporation

# Example: Reviving Dead Data



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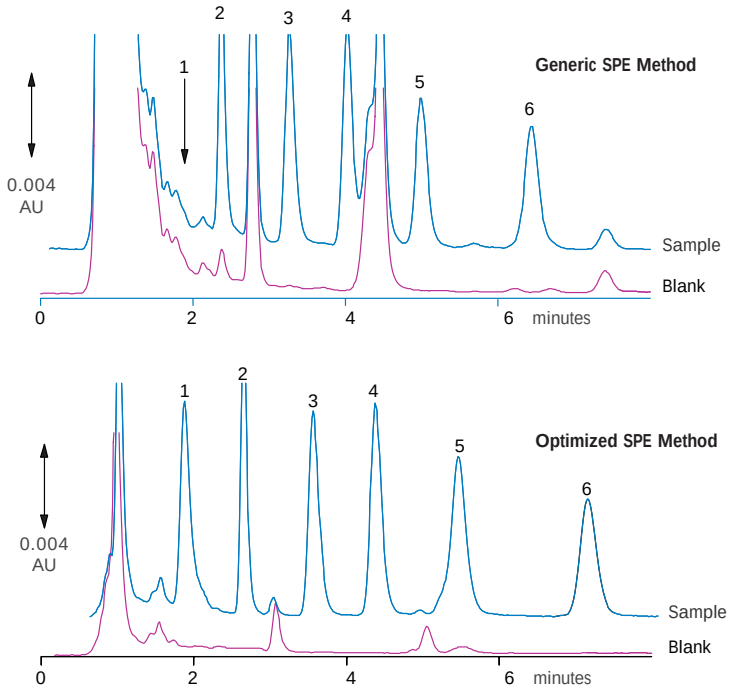
***Meet Selectivity and Sensitivity Goals***

Since the Oasis™ HLB sorbent is stable from pH 1 to 14 and unaffected by common organic solvents, you may take advantage of many more available options for method optimization (Figure 10). For some applications cleaner extracts are required to achieve higher selectivity and sensitivity with traditional ultraviolet or fluorescence detectors. Since retention by the Oasis™ HLB sorbent is very predictable, a cleaner extract can be achieved by simply manipulating the organic concentration and the pH (Figure 11). This procedure (Figure 10) allows compounds to be retained in a non-ionized or more lipophilic form in order to remove interferences with a higher concentration of organic solvent. The pH is then changed to allow the compound to be eluted in the charged or more hydrophilic state, without increasing the percent of organic solvent.

Figure 11

**Meeting the Background Challenge of UV Detectors: Analysis of Tricyclic-Antidepressants in Porcine Plasma**




| | | | |
|---|---|---|---|
| Column: | SymmetryShield™ $RP_8$, 3.5 µm, 4.6 x 75 mm | | |
| Guard Column: | Sentry™ Guard Column SymmetryShield™ $RP_8$, 5 µm | | |
| Temperature: | 29 °C | | |
| Mobile Phase: | 50 mM phosphate, pH 7: methanol (33.6:66.4) | | |
| Detection: | UV at 254 nm | | |
| Flow Rate: | 1.4 mL/min | | |
| Injection Volume: | 140 µL (Generic Method) or 110 µL (Optimized Method) | | |
| Plasma Extracts: | 1. Nordoxepin | 2. Nortriptyline | 3. Doxepin (IS) |
| Spiked at 500 ng/mL | 4. Imipramine | 5. Amitriptyline | 6. Trimipramine |

**Details of Optimized Method**

*Wash 1:* 2% ammonium hydroxide in 5% methanol
*Wash 2:* 2% ammonium hydroxide in 65% methanol
*Wash 3:* 2% acetic acid in 5% methanol
*Elute:* 65% methanol in water or omit wash 3 and elute with 2% acetic acid in 65% methanol

These chromatograms were originally exported from a Waters 860 ExpertEase™ system [VAX] as HGL files, then placed into Freelance documents [PC].

Once converted to **PS**, **PDF**, then **EPS**, line color & thickness were changed, extra objects were removed, labels added, & the final **EPS** files were scaled to fit.

Design Tools: QuarkXPress, Mac OS

Chromatography: Pamela Iraneta

Brochure Design: Susan Hamant

NOTE: *quality of original is preserved, but cannot be improved.*

# Illustration Design Principles



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These three extraordinary volumes written by Prof. Edward R. Tufte [Yale; published by Graphics Press, Box 430, Cheshire, CT, 06410] contain many suggestions on how to display information.

I urge you to read:

1. *The Visual Display of Quantitative Information* [1983]
2. *Envisioning Information* [1990]
3. *Visual Explanations:* *Images and Quantities, Evidence and Narrative* [1997]

Some of Tufte's key principles which I have tried to follow here:

- "Design Strategy of *the smallest effective difference:*
  Make all visual distinctions as subtle as possible,
  but still clear and effective." [#3, p. 73]
- "Graphics *reveal* data."
  "... encourage the eye to compare different pieces of data"
  [#1, p. 3]

# Design Principles, continued



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- "Graphics do not become attractive and interesting through the addition of ornamental hatching and false perspective to a few bars. Chartjunk can turn bores into disasters, but it can never rescue a thin data set."
- "The best designs ... are *intriguing and curiosity-provoking*, drawing the viewer into the wonder of the data."
- "Forgo chartjunk, including moiré vibration, the grid, and the duck." [#1, p. 121]
- "Graphical elements look better together when their relative proportions are in balance." [#1, p. 184]
- "Lines in data graphics should be thin." [#1, p. 185]
- "• If the nature of the data suggests the shape of the graphic, follow that suggestion."

  "• Otherwise, move toward horizontal graphics about 50 percent wider than tall..." [#1, p. 190]

# Final Thoughts



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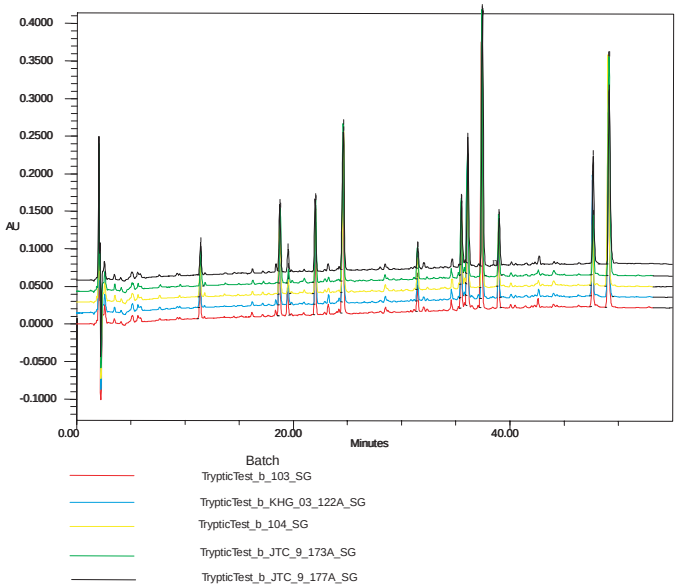
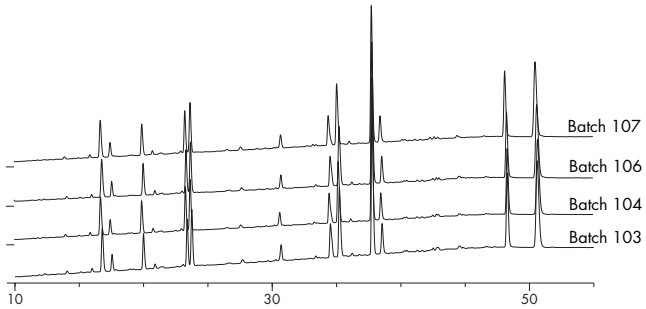
*You be the judge:* [Compare Visual Displays on Pages 4 & 12]

*Which graphic most clearly conveys the message of reproducibility?*




*It Works:* *The illustrations on pages 4, 13, 14, 15, 17 & above left were created using the* ***New Postscript Process*** *described on page 16 & then placed in this ClarisDraw [Mac OS] file as EPS images.*

*We hope this poster inspires you to create superior graphics & exciting, thought-provoking, visual displays of information.*

# Postlude: Recommendations



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- Presentation software is ***not*** recommended: *Freelance* [WIN OS only] & *Powerpoint* [WIN & Mac OS] each have graphics limitations, including import & export formats, color support. Neither could have been used to create this poster.
- Print presentations to **PS**, then distill to **PDF**

  **Advantages:** PDF files can be secured & they:
  - are smaller, ideal for web & email distribution
  - can be run as presentations & slide shows
  - have zoom in & out capability
  - cross more OS platforms [WIN, Mac, Unix]
  - contain fully indexable & searchable text

# Summary: Accomplishments



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*A simple way to prepare publication–ready chromatograms has been devised which:*

- Uses off-the-shelf software
- Crosses software & computer OS platforms
- Produces highest quality, scalable illustrations

*An elegant, easy way to resurrect dead-end illustrations has been discovered which:*

- Uses some unique "hidden" capabilities of Postscript & Adobe software across OS platforms
- Maintains quality; permits modification & scaling

# Gracias Amigos



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I wish to thank especially:

- Dr. Judy Carmody: [at right]

  She graciously exported from her Millennium$^{32}$ runs the chromatographic data which were used to devise & illustrate the plotting procedures described herein. Then she kindly documented her raw data export procedures & taught her colleagues how to do it.

- Those colleagues

  [for their chromatography]

  & the outside designers & agencies

  [for their document layout & PDF files]:

  named on the respective examples herein.

# Want More Information?



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Please leave your *name*, *address*, & *email address*

[on business card or **PRINTED LEGIBLY** on a piece of paper] in the large *envelope provided*.

Please *indicate* which option [**A**, **B** or **C**] you prefer:

**A**. a ***paper*** copy of this poster by mail

**B**. a ***PDF*** copy of this poster via email attachment [indicate whether your client is a PC or a MAC]

**C**. an ***email notification*** of how & when to view, or download, a PDF copy of this poster through the Waters Applications Library at http://www.waters.com