

Global **RESTEK** Advantage

vol. 082

Increase Sample Throughput for Complex Drinking Water Pesticides

Using Rtx[®]-CLPesticides and Rtx[®]-CLPesticides2 Capillary Columns

- Optimized conditions cut analysis time in half, for higher sample throughput.
- Unique selectivity fully resolves complex compound list.
- Meets all method QA requirements, reducing rework.

With the advent of modern agriculture, and its vast selection of chemical pest control measures, the farming community has made significant increases in productivity and efficiency. Crop yield per acre is at an all time high, due in part to the role of pesticides and herbicides in mitigating the devastating effects of many plant and insect pests.¹ However, the use of these chemicals can have drawbacks, including surface and ground water contamination. EPA Methods, such as 508.1, are used to monitor pesticides and herbicides in drinking and ground water. The optimized dual column method shown here satisfies all method requirements in half the analysis time, significantly improving sample throughput.

Continued on page 2.



Chromatography Products

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1 (of 8) 2008-2Global

Increase Sample Throughput for Complex Drinking Water Pesticides

Continued from page 1.

EPA Method 508.1 includes many of the components as Method 505, a similar GC/ECD method, but also contains several others, expanding the list to 38 compounds. This method calls for solid phase extraction and extract concentration, followed by analysis using a GC/ECD system. In order to increase sample throughput, an optimized method was developed using a dual column configuration with the Rtx®-CLPesticides/Rtx®-CLPesticides2 column pair. These columns, used under the conditions shown, offer a unique selectivity that allows the target analytes to be resolved in approximately half the analysis time of the original method (Figure 1). There was one coelution on the primary column, but these compounds were separated on the second column. Both columns easily passed the comprehensive system performance criteria adapted from 508.1 (Table I).²

In conclusion, due to the complexity of the compound list in Method 508.1, a very high degree of selectivity is required of the capillary column in order to achieve adequate resolution of all target analytes in a reasonable time. The optimized dual column method shown here offers a significantly faster analysis time, while maintaining excellent resolution of challenging drinking water pesticides and herbicides.

References

1. <http://www.usda.gov/nass/pubs/trackrec/track00a.htm#principal>
2. US EPA Method 508.1, James W Eichelberger Rev 1.0 1994.

Conditions for Figure 1

Column: Rtx®-CLPesticides2, 30m, 0.32mm ID, 0.25µm (cat.# 11324) and Rtx®-CLPesticides, 30m, 0.32mm ID, 0.32µm (cat.# 11141) with 5m x 0.32mm ID Rxi® deactivated guard tubing (cat.# 10039), connected using Universal "Y" Press-Tight® Connector (cat.# 20405-261)

Sample: 50ng/mL 508.1 Calibration Mix #1 (cat.# 32094), 100ng/mL 508.1 Calibration Mix #2 (cat.# 32095), 100ng/mL 508.1 Calibration Mix #3 (cat.# 32096), 50ng/mL 508.1 Internal Standard (cat.# 32091), 250ng/mL 508.1 Surrogate (cat.# 32092), 500ng/mL Atrazine (cat.# 32208), 500ng/mL Simazine (cat.# 32236) in ethyl acetate

Inj.: 2µL splitless (hold 0.75 min.), 4mm cyclo double gooseneck liner (cat.# 20896)

Inj. temp.: 250°C

Carrier gas: helium, constant flow

Linear velocity: 26cm/sec. @ 80°C

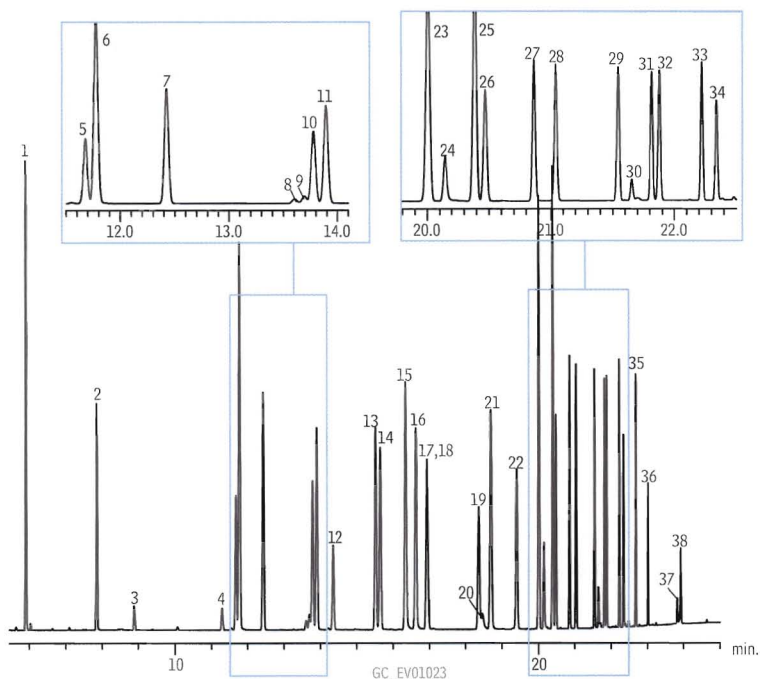
Oven temp.: 80°C (hold 0.5 min.) to 155°C (hold 1 min.) @ 19°C/min. to 210°C @ 4°C/min. to 310°C (hold 0.5 min.) @ 25°C/min.

Detector temp.: ECD @ 325°C

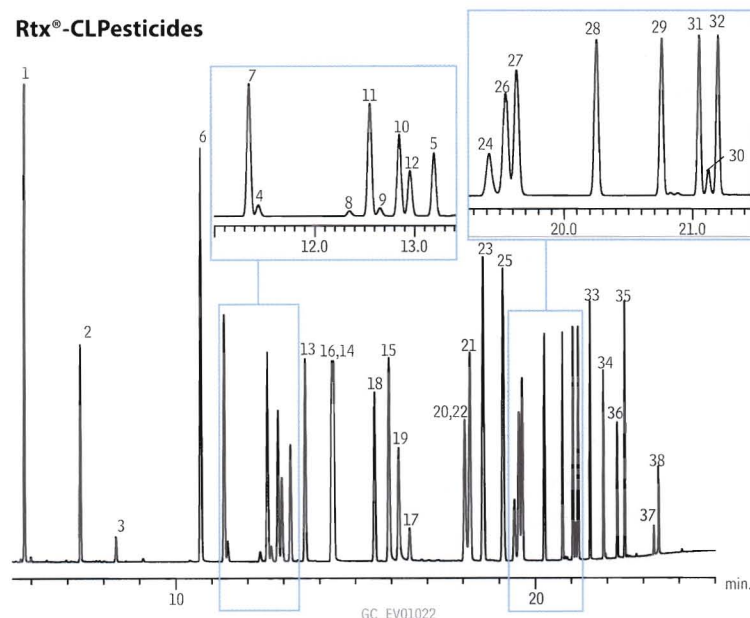
Figure 1 Resolve all critical pairs using Rtx®-CLPesticides and Rtx®-CLPesticides2 columns.

- | | | |
|----------------------------------|-------------------------------|------------------------|
| 1. hexachlorocyclopentadiene | 14. heptachlor | 27. 4,4'-DDE |
| 2. etridiazole | 15. chlorothalonil | 28. dieldrin |
| 3. chlorneb | 16. metribuzin | 29. endrin |
| 4. propachlor | 17. alachlor | 30. chlorobenzilate |
| 5. trifluralin | 18. aldrin | 31. 4,4'-DDD |
| 6. hexachlorobenzene | 19. 4,4'-dibromobiphenyl (SS) | 32. endosulfan II |
| 7. α-BHC | 20. metachlor | 33. 4,4'-DDT |
| 8. simazine | 21. DCPA | 34. endrin aldehyde |
| 9. atrazine | 22. heptachlor epoxide | 35. endosulfan sulfate |
| 10. pentachloronitrobenzene (IS) | 23. γ-chlordane | 36. methoxychlor |
| 11. γ-BHC | 24. cyanazine | 37. cis-permethrin |
| 12. β-BHC | 25. α-chlordane | 38. trans-permethrin |
| 13. δ-BHC | 26. endosulfan I | |

Rtx®-CLPesticides2



Rtx®-CLPesticides



Satisfy all method requirements in half the time!

Table I Rtx®-CLPesticides and Rtx®-CLPesticides2 columns easily pass EPA Method 508.1 performance criteria.

Test/Requirement	Analyte	Concentration (ppb)	Rtx®-CLPesticides2	Rtx®-CLPesticides
Inertness (breakdown <20%)	endrin	50	0.9%	1.4%
Inertness (breakdown <20%)	4,4'-DDE	100	1.0%	1.1%
Sensitivity (S/N>3)	chlorpyrifos	2	12.0	6.2
Chromatographic performance (0.8<PGF<1.15)	DCPA	50	1.03	1.06
Column performance (resolution>0.50)	chlorothalonil	50	9.9	26.8
Column performance (resolution>0.50)	gamma-BHC	40	9.9	26.8

Rxi® Guard/Retention Gap Columns (fused silica)

Nominal ID	Nominal OD	5-Meter	5-Meter/6-pk.	10-Meter	10-Meter/6-pk.
0.25mm	0.37 ± 0.04mm	10029	10029-600	10059	10059-600
0.32mm	0.45 ± 0.04mm	10039	10039-600	10064	10064-600
0.53mm	0.69 ± 0.05mm	10054	10054-600	10073	10073-600

Universal "Y" Press-Tight® Connectors

Description	ea.	3-pk.
Universal "Y" Press-Tight Connector	20405	20406
Deactivated Universal "Y" Press-Tight Connector	20405-261	20406-261
Siltek Treated Universal "Y" Press-Tight Connector	20485	20486

Rtx®-CLPesticides Columns (fused silica)

ID	df (μm)	temp. limits	length	cat. #
0.32mm	0.32	-60 to 320/340°C	30-Meter	11141

508.1 Calibration Mix #1 (17 components)

aldrin	endosulfan I
α-BHC	endosulfan II
β-BHC	endosulfan sulfate
δ-BHC	endrin
γ-BHC (lindane)	endrin aldehyde
4,4'-DDD	heptachlor
4,4'-DDE	heptachlor epoxide (isomer B)
4,4'-DDT	methoxychlor
dieldrin	
500μg/mL each in ethyl acetate, 1mL/ampul	
cat. # 32094	

508.1 Calibration Mix #2 (11 components)

chlorobenzilate	hexachlorobenzene
α-chlordane	cis-permethrin*
γ-chlordane	trans-permethrin*
chlornelb	propachlor
DCPA (Dacthal®)	trifluralin
etridiazole	
500μg/mL each in ethyl acetate, 1mL/ampul	
cat. # 32095	

*1000μg/mL total permethrin. Exact content of each isomer listed on certificate of analysis.

508.1 Calibration Mix #3 (8 components)

alachlor	hexachlorocyclopentadiene
atrazine	metolachlor
chlorthalonil	metribuzin
cyanazine	simazine
500μg/mL each in ethyl acetate, 1mL/ampul	
cat. # 32096	

Rtx®-CLPesticides2 Columns (fused silica)

ID	df (μm)	temp. limits	length	cat. #
0.32mm	0.25	-60 to 320/340°C	30-Meter	11324

508.1 Internal Standard

pentachloronitrobenzene
100μg/mL in ethyl acetate, 1mL/ampul
cat. # 32091

508.1 Surrogate

4,4'-dibromobiphenyl
500μg/mL in ethyl acetate, 1mL/ampul
cat. # 32092

Atrazine

1,000μg/mL in acetone, 1mL/ampul
cat. # 32208

Simazine

1,000μg/mL in acetone, 1mL/ampul
cat. # 32236

Splitless Liners for Agilent

ID* x OD & Length	qty.	cat. #
Cyclo Double Gooseneck (4mm)		
4.0mm x 6.5mm x 78.5mm	5-pk.	20896

*Nominal ID at syringe needle expulsion point.

Resprep™-C18 SPE Disks

Description	qty.	cat. #
Resprep™-C18 47mm SPE Disks	20-pk.	24004



Fast, Simple Sample Cleanup

Using QuEChERS SPE Tubes

- Achieve a four-fold increase in sample throughput.
- Significantly reduce material costs.
- Convenient, ready to use centrifuge tubes with ultra pure, pre-weighed adsorbent mixtures.



Quick, Easy, Cheap, Effective, Rugged, and Safe, the QuEChERS (“catchers”) method for extracting pesticides from food is based on research by the US Department of Agriculture.¹ In addition to using less solvent and materials versus conventional SPE methods, QuEChERS employs a novel and much quicker dispersive solid phase extraction cleanup (dSPE). QuEChERS methods, including an AOAC Official Method² and modifications to the methods, have been posted on the Internet.³ These methods have several basic steps in common:

Step 1: Sample preparation and extraction—Commodities are uniformly comminuted. Acetonitrile solvent is added for a shake extraction. Salts, acids and buffers may be added to enhance extraction efficiency and protect sensitive analytes. Surrogate standards can be added to monitor extraction efficiencies.

Step 2: Extract cleanup – A subsample of solvent extract is cleaned up using dSPE, a key improvement incorporated in the QuEChERS technique. Small polypropylene centrifuge tubes are prefilled with precise weights of MgSO_4 and SPE adsorbents to remove excess water and unwanted contaminants from the extracted samples. After agitation and centrifugation, the cleaned extracts are ready for analysis.

Step 3: Sample analysis – Samples may be pH adjusted to protect sensitive pesticides and/or solvent-exchanged to improve analysis by either GC/MS or LC/MS. Internal standards can be added.

Table I Modified mini-multiresidue QuEChERS for pesticides from strawberries.

Sample preparation and extraction	
Sample:	10g of strawberries were homogenized and placed in a 50mL PTFE centrifuge tube
Solvent:	10mL of acetonitrile were added to homogenate Shake for 1 minute, until uniform
Salts:	4.0g MgSO_4 (powder or granular) 1.1.0g NaCl 1.0g trisodium citrate dihydrate 0.5g disodium hydrogen citrate sesquihydrate Salts were added and vigorously shaken for 1 minute. Sample was centrifuged and the supernatant removed for cleanup. Pesticides standards (200ng/mL) were spiked in at this point.
Sample extract cleanup	
QuEChERS tubes:	1mL of supernatant from the previous step was placed into several 2mL polypropylene centrifuge tubes, each containing one of the following adsorbent mixes: A. 50mg PSA + 150mg MgSO_4 (cat.# 26124) B. 50mg PSA + 150mg MgSO_4 + 50mg C18 (cat.# 26125) C. 50mg PSA + 150mg MgSO_4 + 50mg GCB (cat.# 26123)
Cleanup:	Samples were shaken with the adsorbents for 30 seconds (carbon for 2 minutes), then centrifuged to produce a clear supernatant for GC/MS analysis.
Internal standard:	Pentachloronitrobenzene in a formic acid solution, pH 5.
PSA—primary and secondary amine exchange material.	
GCB—graphitized carbon black	

Table II Instrument conditions.

Column:	Rtx®-CLPesticides2 20m, 0.18mm ID, 0.14 μm (cat.# 42302)
Sample:	custom pesticide mix 200 $\mu\text{g/mL}$ each pesticide, internal standards: 8140-8141 ISTD, 1000 $\mu\text{g/mL}$ (cat.# 32279), 508.1 ISTD 100 $\mu\text{g/mL}$ (cat.# 32091), triphenylphosphate 1000 $\mu\text{g/mL}$ (cat.# 32281) 1.0 μL splitless (hold 1 min.)
Inj.:	
Inj. temp.:	250°C
Carrier gas:	helium
Flow rate:	constant linear velocity @ 40cm/sec
Oven temp.:	40°C (hold 1 min.) to 320°C @ 12°C/min.
Det:	Shimadzu GCMS-QP2010 Plus
Transfer line temp.:	300°C
Ionization:	Electron ionization
Mode:	Selected ion monitoring



QuEChERS methods are convenient, rugged methods that simplify extract cleanup, reduce material costs, and improve sample throughput. Here we demonstrate the effectiveness of QuEChERS sample cleanup using a multiresidue analysis of pesticides on strawberries.

Experimental

Strawberry extracts were prepared, spiked, and dSPE treated according to Table I. Analytical conditions are presented in Table II.

One microliter splitless injections of the extracts were performed by a Shimadzu AOC-20i autosampler using “mid” injection speed into a Shimadzu QP-2010 Plus GC-MS system operated under the conditions in Table II.

Rtx®-CLPesticides2 Columns (fused silica)

ID	df (μm)	temp. limits	length	cat. #
0.18mm	0.14	-60 to 310/330°C	20-Meter	42302

Results and Discussion

Primary and secondary amine exchange material (PSA) is the base sorbent used for dSPE cleanup of QuEChERS fruit and vegetable extracts because it removes many organic acids and sugars that might act as instrumental interferences.

A pesticide-spiked strawberry extract (200ng/mL) subjected to dSPE with PSA was used to generate one-point calibration curves. Spiked strawberry extracts subjected to additional dSPE sorbents were analyzed and the results versus PSA dSPE are shown as percent recoveries in Table III. C18 is suggested for use when samples might contain fats; not an issue for a strawberry extract, but it was important to verify that gross losses of more hydrophobic pesticides (e.g. Endrin and DDT) would not occur. GCB is used to remove pigments, and when treated, the pink/red strawberry extract became clear. However, GCB can also have a negative effect on certain pesticides, especially those that can assume a planar shape like chlorothalonil and thiabendazole.

Restek dSPE products in a variety of standard sizes and formats make QuEChERS even simpler. The centrifuge tube format, available in 2mL and 15mL sizes, contains magnesium sulfate (to partition water from organic solvent) and a choice of SPE sorbents, including PSA (to remove sugars and fatty acids), C18 (to remove nonpolar interferences such as fats), and GCB (to remove pigments and sterols). Custom products also are available by request. If you are frustrated by the time and cost involved with your current approach to pesticide sample cleanup, we suggest you try this simple and economical new method.

References

1. Michelangelo Anastassiades, Steven J. Lehotay, Darinka Štajnbaher, Frank J. Schenck. "Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and Dispersive Solid-Phase Extraction for the Determination of Pesticide Residues in Produce." *J. AOAC International*, 2003, vol. 86(22), pp.412-431.
2. AOAC Official Method 2007.01, "Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate."
3. <http://www.quechers.com/>
References not available from Restek

Table III Pesticide percent recoveries in strawberry extracts treated with C18 or GCB dSPE, relative to PSA only.

Rt (min.)	pesticide	CAS Number	action/Use	classification	C18*	GCB**
9.50	Dichlorvos	62-73-7	Insecticide	Organophosphorus	111	116
9.67	Methamidophos	10265-92-6	Insecticide	Organophosphorus	105	107
11.75	Mevinphos	7786-34-7	Insecticide	Organophosphorus	112	130
12.02	o-Phenylphenol	90-43-7	Fungicide	Unclassified	106	97
12.14	Acephate	30560-19-1	Insecticide	Organophosphorus	128	147
13.89	Omethoate	1113-02-6	Insecticide	Organophosphorus	120	119
14.74	Diazinon	333-41-5	Insecticide	Organophosphorus	108	127
14.98	Dimethoate	60-51-5	Insecticide	Organophosphorus	124	151
15.69	Chlorothalonil	1897-45-6	Fungicide	Organochlorine	125	13
15.86	Vinclozolin	50471-44-8	Fungicide	Organochlorine	102	98
16.21	Metalaxyl	57837-19-1	Fungicide	Organonitrogen	105	117
16.28	Carbaryl	63-25-2	Insecticide	Carbamate	114	111
16.60	Malathion	121-75-5	Insecticide	Organophosphorus	124	160
16.67	Dichlofluanid	1085-98-9	Fungicide	Organohalogen	122	103
17.51	Thiabendazole	148-79-8	Fungicide	Organonitrogen	88	14
17.70	Captan	133-06-2	Fungicide	Organochlorine	88	91
17.76	Folpet	133-07-3	Fungicide	Organochlorine	108	63
18.23	Imazalil	35554-44-0	Fungicide	Organonitrogen	115	95
18.39	Endrin	72-20-8	Insecticide	Organochlorine	104	101
18.62	Myclobutanil	88671-89-0	Fungicide	Organonitrogen	119	114
19.07	4,4-DDT	50-29-3	Insecticide	Organochlorine	102	95
19.22	Fenhexamid	126833-17-8	Fungicide	Organochlorine	118	77
19.40	Propargite 1	2312-35-8	Acaricide	Organosulfur	110	95
19.43	Propargite 2	2312-35-8	Acaricide	Organosulfur	121	114
19.75	Bifenthrin	82657-04-3	Insecticide	Pyrethroid	106	81
20.04	Dicofol	115-32-2	Acaricide	Organochlorine	98	54
20.05	Iprodione	36734-19-7	Fungicide	Organonitrogen	118	90
20.21	Fenpropathrin	39515-41-8	Insecticide	Pyrethroid	113	96
21.32	cis-Permethrin	52645-53-1	Insecticide	Pyrethroid	106	65
21.47	trans-Permethrin	51877-74-8	Insecticide	Pyrethroid	109	71
23.74	Deltamethrin	52918-63-5	Insecticide	Pyrethroid	97	52

*50mg PSA, 50mg C18, **50mg PSA, 50mg GCB
% recovery = $\frac{\text{RRF C18 or GCB}}{\text{RRF PSA}} \times 100$

QuEChERS SPE Tubes

AOAC Method 2007.1	Benefits/Uses	qty.	cat#
2mL QuEChERS SPE Micro-Centrifuge Tube Contains 150mg Magnesium Sulfate and 50mg PSA	Cleanup of agricultural produce extracts, 1mL sample volume.	100-pk.	26124
2mL QuEChERS SPE Micro-Centrifuge Tube Contains 150mg Magnesium Sulfate, 50mg PSA, and 50mg Graphitized Carbon	Cleanup of 1mL sample extract with residual pigments and sterols.	100-pk.	26123
2mL QuEChERS SPE Micro-Centrifuge Tube Contains 150mg Magnesium Sulfate, 50mg PSA, and 50mg C18	Cleanup of 1mL sample extract with residual fat.	100-pk.	26125
15mL QuEChERS SPE Centrifuge Tube Contains 900mg Magnesium Sulfate, 300mg PSA, and 150mg Graphitized Carbon	Cleanup of 6mL sample extract with residual pigments and sterols.	50-pk.	26126

PSA—primary and secondary amine exchange material.



FREE Sample Packs Available!

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Easy Transfer of HPLC Methods to UHPLC

Using Fully Scalable Pinnacle™ DB Columns

- Methods on Pinnacle™ DB columns are easily transferred from 3 and 5µm to 1.9µm, allowing faster analysis without losing separation quality.
- Pinnacle™ DB columns are 100% Restek manufactured—from base silica to final packed column.
- Restek offers the widest selection of stationary phases for UHPLC—more choices mean better selectivity for your analytes.

Ultra High Pressure Liquid Chromatography (UHPLC) is a rapidly growing technique that produces significantly faster analysis times compared to conventional HPLC. While transferring HPLC methods to UHPLC can increase sample throughput, comparable method parameters must be used to maintain equivalent separations. Here we review which column properties and operating conditions should remain consistent and which need to be optimized in order to maintain selectivity.

In this example, we will perform a scale-down method transfer for sulfonamides (Figure 1). For optimal selectivity and faster analysis times, we used a Pinnacle™ DB Biphenyl stationary phase for this application (Figure 2). When performing a scale-down procedure, column pore size, carbon load, and support material must remain the same. Changes to other parameters can be made using a few simple calculations. Let's go through them sequentially.

Adjusting Column Size

The first calculation determines the appropriate column length. Keeping the same column length while decreasing the particle size increases the number of theoretical plates. Therefore, column length can be shortened without losing resolution. By adjusting the column length properly, using Equation 1, we can maintain the same separation.

Adjusting Injection Volume

Once we have determined the proper column length, we can calculate injection volume. Decreasing the column internal diameter and length decreases the overall column volume and sample capacity. Therefore, we must alter the injection volume as described in Equation 2. Note that since overall column volume has decreased, it is important to match the sample solvent to the starting mobile phase composition. Mismatched sample solvents can cause irreproducible retention times, efficiencies, and even changes in selectivity.

Adjusting Flow rate

Next, flow rate must be adjusted to maintain comparable linear velocity through a column with smaller internal diameter. To maintain the same linear velocity (which is important in maintaining efficiencies), flow rates must be decreased. Also, since smaller particle sizes give rise to higher optimal linear velocities, isocratic flow rates should be calculated with particle size taken into account. In this example, a gradient elution was used and, therefore, particle size was not included in the equation. Equation 3 can be used to estimate the adjusted flow rate needed for equivalent chromatography. Also, note that since <2µm particle sizes are less affected by flow rate, faster flow rates can be used in isocratic systems without detrimental effects on peak efficiency.

Adjusting Time Program

After determining the proper column length, injection volume, and flow rate, we can calculate the time needed for gradient or step elutions. As an analytical method is scaled down, the time program also needs to be scaled down to keep the phase interactions the same. Time can be adjusted using Equation 4.

Figure 1 Chemical structures for example sulfonamides.

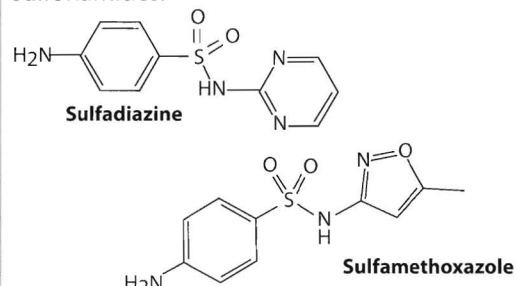
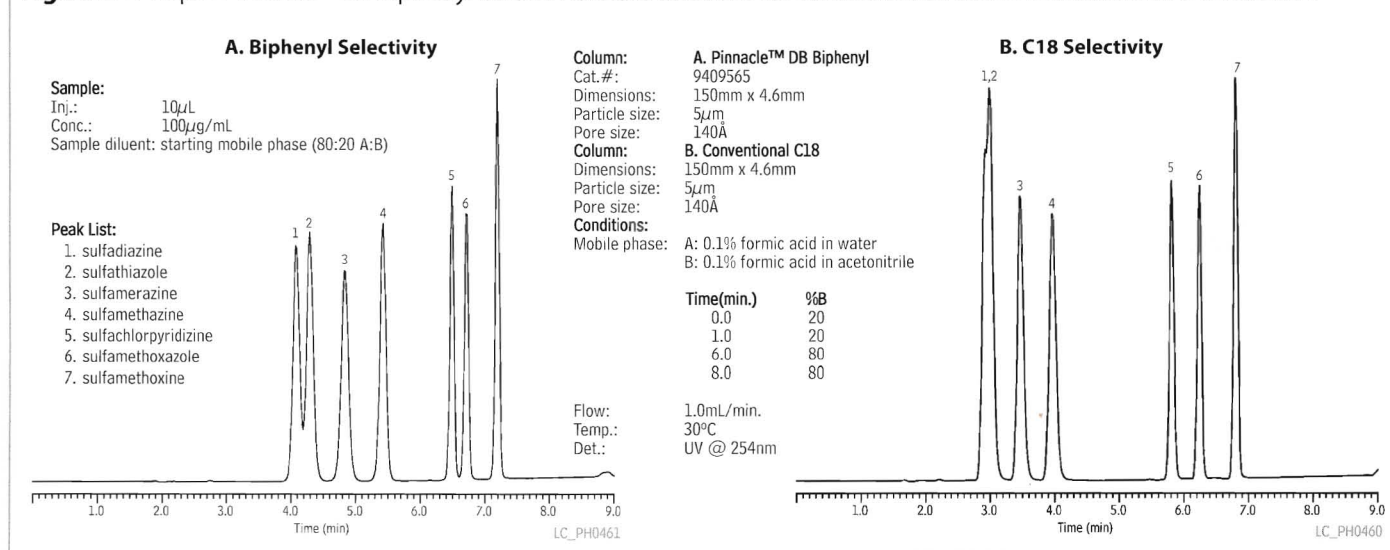


Figure 2 A 1.9µm Pinnacle™ DB Biphenyl column is more selective for sulfonamides than a conventional C18 column.



How Many Plates?

Winners of Restek's Column Contest from the 32nd International Symposium on Capillary GC Announced

The *International Symposium on Capillary GC* is one of the leading symposia on capillary separation technology in the world. Restek contributed to this event with many technical posters and papers, but we also had time for a little fun!

Prof. Marriot challenging his brain...



At Restek's booth, a game was played where the participant had to guess the plate number of a GC column and an LC column. The prize was a free GC or LC column. The GC column chosen for the challenge, was a 20 m x 0.18mm Rxi-5 Sil MS. The LC column was a 5 cm x 2.1 mm. 1.9 um Pinnacle DB. Many visitors made their guess by looking at the chromatogram or calculating efficiency from column dimensions.

The winner on the GC column was Prof. Philip Marriot, RMIT University, Melbourne, Australia. His estimation of 112,000 theoretical plates was within 2% of the real value!

The winner for the closest plate number guess for the LC column was Pavel Karasek, from the Institute of Analytical Chemistry, Brno, Czech Republic.

Congratulations to both scientists!

Visit http://www.restek.com/ts_riva2008.asp for electronic copies of Restek's posters and papers presented at the 32nd International Symposium on Capillary GC.

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7 (of 8) 2008-2 Global

Equation 1 Adjusted column length can easily be calculated when scaling from HPLC to UHPLC.

$$L_C^2 = \frac{L_C^1 \cdot dp_2}{dp_1}$$

Example:

$$L_C^2 = \frac{150\text{mm} \cdot 1.9\mu\text{m}}{5\mu\text{m}}$$

$$L_C^2 = 57\text{mm}$$

L_C = Column Length
 dp = Particle Size

Equation 2 Changing column dimensions requires an adjusted injection volume.

$$V_{I^2} = V_{I^1} \cdot \left(\frac{d_C^2 \cdot L_C^2}{d_C^1 \cdot L_C^1} \right)$$

Example:

$$V_{I^2} = 10\mu\text{L} \cdot \left(\frac{2.1\text{mm}^2 \cdot 50\text{mm}}{4.6\text{mm}^2 \cdot 150\text{mm}} \right)$$

$$V_{I^2} = 0.69\mu\text{L}$$

V_I = Injection Volume
 L_C = Column Length
 d_C = Column Diameter

Equation 3 Changing column internal diameter requires using an adjusted flow rate.

$$F_{C^2} = \left(\frac{d_C^2}{d_C^1} \right)^2 \cdot F_{C^1}$$

Example:

$$F_{C^2} = \left(\frac{2.1\text{mm}}{4.6\text{mm}} \right)^2 \cdot 1.0\text{ml/min.}$$

$$F_{C^2} = 0.208\text{ ml/min.}$$

F_C = Column Flow
 d_C = Column Diameter

Equation 4 When scaling down a gradient method, the time program needs to be adjusted.

$$t_{G^2} = t_{G^1} \cdot \left(\frac{F_{C^1}}{F_{C^2}} \right) \cdot \left(\frac{d_C^2 \cdot L_C^2}{d_C^1 \cdot L_C^1} \right)$$

Example:

$$t_{G^2} = 5\text{ min.} \cdot \left(\frac{1.0\text{ml/min.}}{0.2\text{ml/min.}} \right) \cdot \left(\frac{2.1\text{mm}^2}{4.6\text{mm}^2} \right) \cdot \left(\frac{50\text{mm}}{150\text{mm}} \right)$$

$$t_{G^2} = 1.7\text{ min.}$$

t_G = Gradient Time
 F = Column Flow
 L_C = Column Length
 d_C = Column Diameter

Conclusion

After determining the equivalent conditions for scaling-down the analysis of sulfonamides, we can see that the separations are equivalent, while the analysis time was greatly reduced (Figure 3). By following the procedure described here to ensure that the columns are equivalent, scaling analytical procedures from HPLC to UHPLC can easily be accomplished using Pinnacle™ DB columns.

Pinnacle™ DB Biphenyl Columns (USP L11)

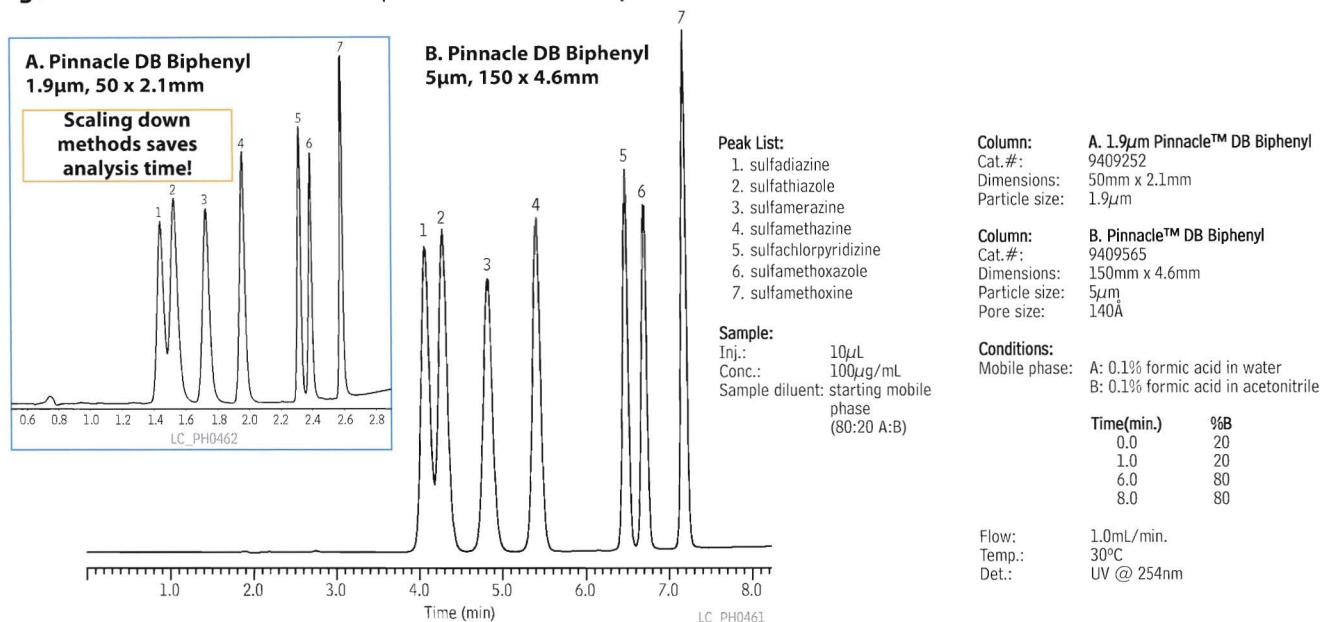
Physical Characteristics:

particle size: 1.9μm or 5μm, endcap: yes
spherical pH range: 2.5 to 7.5
pore size: 140Å temperature limit: 80°C
carbon load: 8%

1.9μm Column, 2.1mm	cat. #
50mm	9409252
5μm Column, 4.6mm	cat. #
150mm	9409565

For other dimensions and guard cartridges for these columns, visit our website at www.restek.com.

Figure 3 Restek's Pinnacle™ DB 1.9μm columns can easily be scaled from HPLC to UHPLC and vice versa.



NEW! Waste Overflow Indicator for HPLC Systems

- Avoid messy pooling around mobile phase waste containers.
- Audible alarm instantly alerts user, preventing overflow.
- Compact, battery operated unit.

The new Restek Waste Overflow Indicator will help to keep your mobile phase waste where it belongs—in the waste container! Compact, battery operated unit fits securely on 4-liter solvent bottles and accommodates two waste streams. An audible alarm is given as the solvent waste container approaches capacity, giving you time to empty or change the container. Another innovative design from Restek!

Description	qty.	cat. #
Waste Overflow Indicator for HPLC Systems	kit	26543
Replacement AA Battery for the Waste Overflow Indicator	ea.	26544
Replacement AA Batteries for the Waste Overflow Indicator	3-pk.	26545

For more information about HPLC Accessories, visit us online at www.restek.com/HPLC

