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.

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.

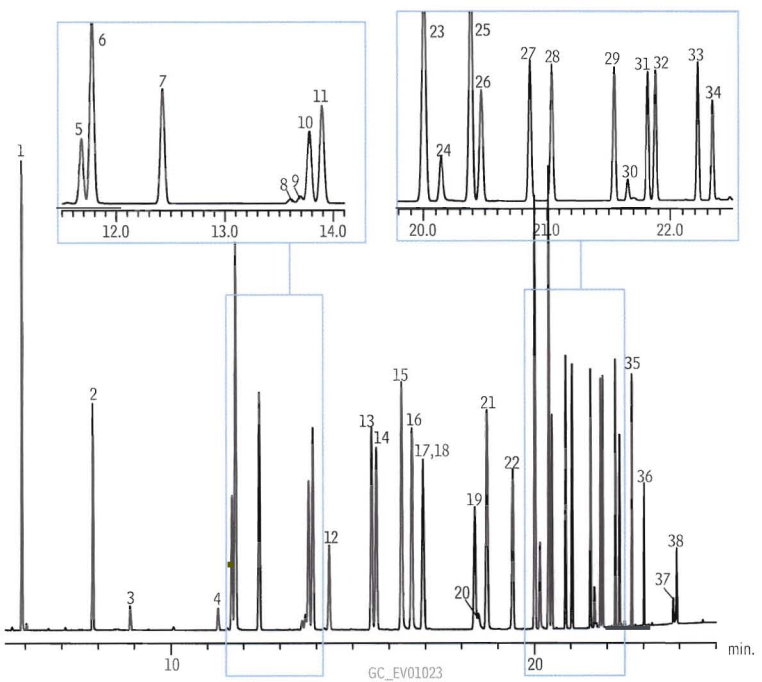
Conditions for Figure 1

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

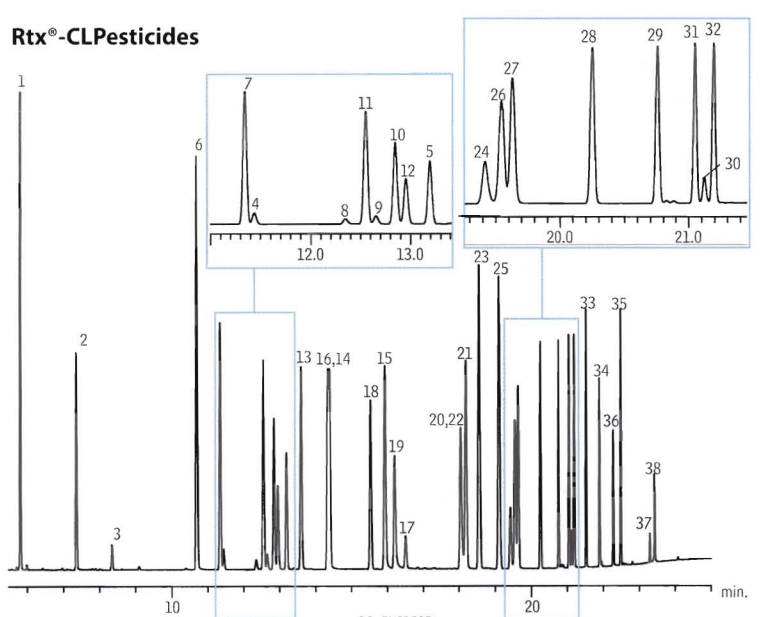
Figure 1 Resolve all critical pairs using Rtx®-CLPesticides and Rtx®-CLPesticides2 columns.

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

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

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 Calibration Mix #1 (17 components)

aldrin
α-BHC
β-BHC
δ-BHC
γ-BHC (lindane)
4,4'-DDD
4,4'-DDE
4,4'-DDT
dieldrin
endosulfan I
endosulfan II
endosulfan sulfate
endrin
endrin aldehyde
heptachlor
heptachlor epoxide (isomer B)
methoxychlor

500µg/mL each in ethyl acetate, 1mL/ampul
cat. # 32094

508.1 Calibration Mix #2 (11 components)

chlorobenzilate
α-chlordane
γ-chlordane
chlorneb
DCPA (Dacthal®)
etridiazole
hexachlorobenzene
cis-permethrin*
trans-permethrin*
propachlor
trifluralin

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
atrazine
chlorthalonil
cyanazine
hexachlorocyclopentadiene
metolachlor
metribuzin
simazine

500µg/mL each in ethyl acetate, 1mL/ampul
cat. # 32096

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, **E**asy, **Ch**eap, **E**ffective, **R**ugged, and **S**afe, 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 Method[2] 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.

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.

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 MgSO4 (powder or granular) 1.1.0g NaCl 1.0g trisodium citrate dihydrate 0.5g disodium hydrogencitrate 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, 0.14µm (cat.# 42302)
Sample:	custom pesticide mix 200µg/mL each pesticide, internal standards: 8140-8141 ISTD, 1000µg/mL (cat.# 32279), 508.1 ISTD 100µg/mL (cat.# 32091), triphenylphosphate 1000µg/mL (cat.# 32281)
Inj.:	1.0µL splitless (hold 1 min.)
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

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

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

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!

To receive your free sample pack, add -248 to the item number. (One sample per customer)

Easy Transfer of HPLC Methods to UHPLC

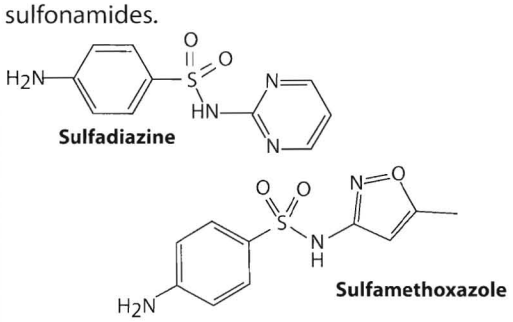
Using Fully Scalable Pinnacle™ DB Columns

- 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.

Figure 1 Chemical structures for example sulfonamides.



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.

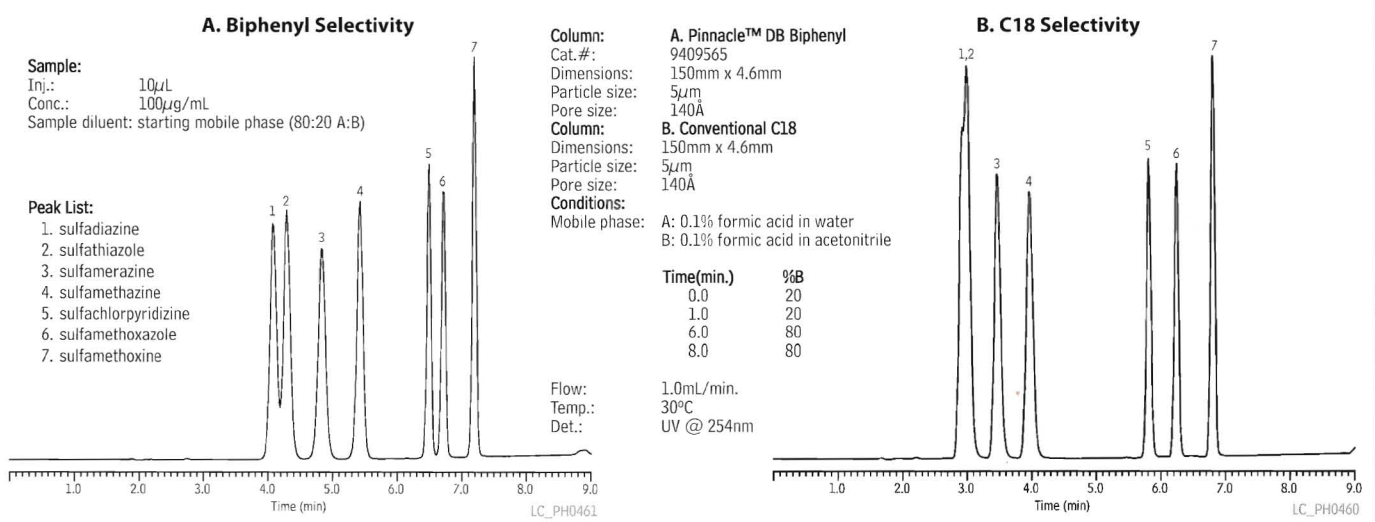
Adjusting Flow rate

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

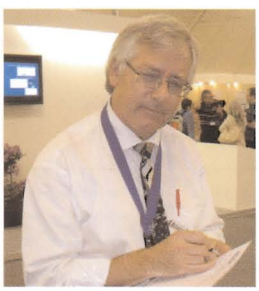


How Many Plates?

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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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.

$$V_{I^2} = V_{I^1} \cdot \left(\frac{d_{c^2}^2 \cdot L_{c^2}}{d_{c^1}^2 \cdot L_{c^1}}\right)$$

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.

$$t_{g^2} = t_{g^1} \cdot \left(\frac{F_{c^1}}{F_{c^2}}\right) \cdot \left(\frac{d_{c^2}^2}{d_{c^1}^2}\right) \cdot \left(\frac{L_{c^2}}{L_{c^1}}\right)$$

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)

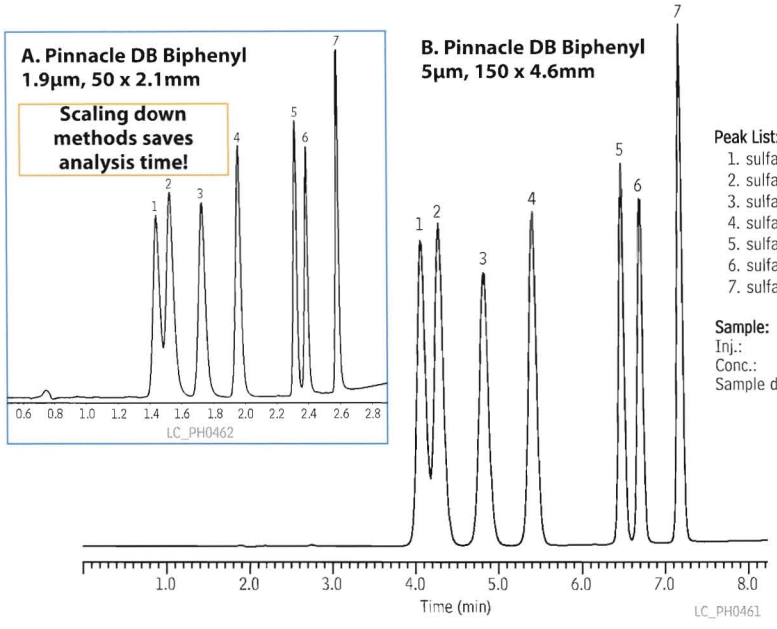
Physical Characteristics:

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

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.



Peak List:
1. sulfadiazine
2. sulfathiazole
3. sulfamerazine
4. sulfamethazine
5. sulfachlorpyridizine
6. sulfamethoxazole
7. sulfamethoxine

Sample:
Inj.: 10µL
Conc.: 100µg/mL
Sample diluent: starting mobile phase (80:20 A:B)

Column: A. 1.9µm Pinnacle™ DB Biphenyl
Cat.#: 9409252
Dimensions: 50mm x 2.1mm
Particle size: 1.9µm

Column: B. Pinnacle™ DB Biphenyl
Cat.#: 9409565
Dimensions: 150mm x 4.6mm
Particle size: 5µm
Pore size: 140Å

Conditions:
Mobile phase: A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile

Time(min.)	%B
0.0	20
1.0	20
6.0	80
8.0	80

Flow: 1.0mL/min.
Temp.: 30°C
Det.: UV @ 254nm

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

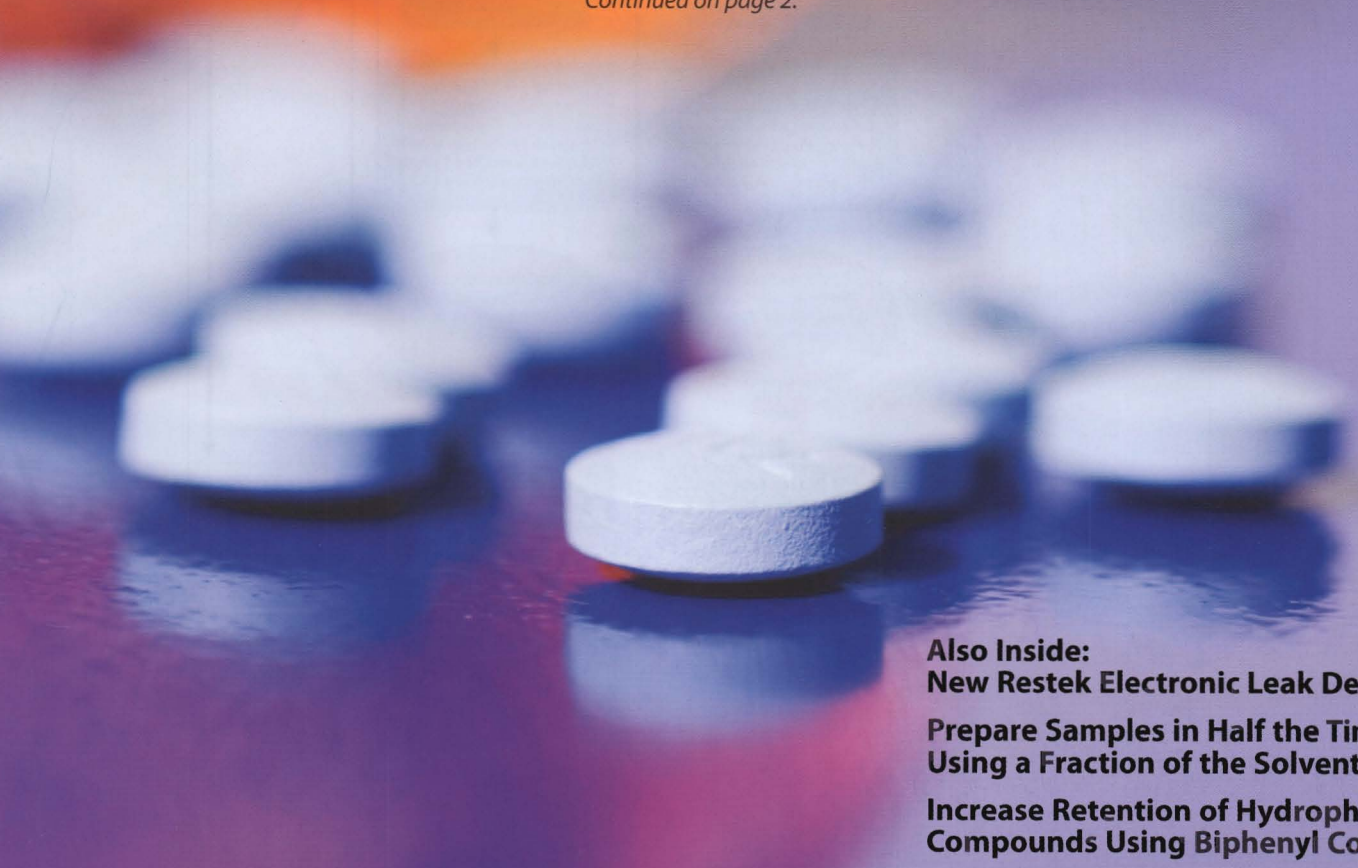
For more information about HPLC Accessories, visit us online at www.restek.com/HPLC

Global Restek Advantage
2009.01

Successfully Implement the Revised USP <467> Method

The USP general chapter <467> Residual Solvents is a widely used compendial method for identifying and quantifying residual solvents when no information is available on what solvents are likely to be present. In an attempt to harmonize with the ICH guidelines, the USP has proposed a more comprehensive method in the current USP 30/NF 25. This revision significantly increases the number of residual solvents to be routinely tested and includes three distinct procedures.[1]

Continued on page 2.



Also Inside:

New Restek Electronic Leak Detector

Prepare Samples in Half the Time Using a Fraction of the Solvent

Increase Retention of Hydrophilic Compounds Using Biphenyl Columns

Contents

Successfully Implement the Revised USP <467> Method

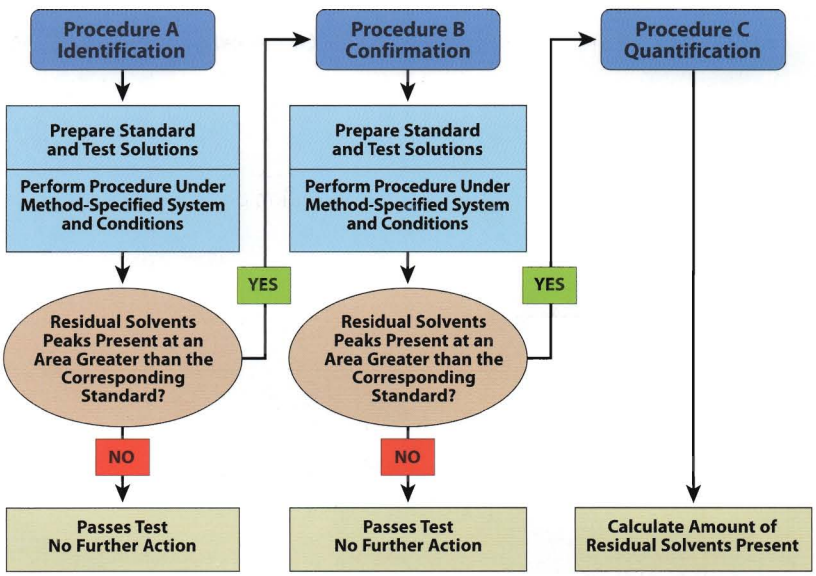
Continued from page 1.

Overview of Method

The revised USP <467> method consists of a static headspace extraction coupled with a gas chromatographic separation and flame ionization detection. In this guide we demonstrate the USP <467> application using two different types of headspace autosamplers. Procedure A was performed using a pressured loop autosampler and transfer line. Procedure B was performed using a heated syringe injection. Either system can be used to meet method requirements.

USP <467> is divided into two separate sections based upon sample solubility: water-soluble and water-insoluble articles. The methodology for both types of articles is similar, but the diluent used in both standard and sample preparations differs based upon the solubility of the test article. The test method consists of three procedures (A, B, and C), that are designed to identify, confirm, and then quantify residual solvents in drug substances and products (Figure 1).

Figure 1 Analytical flow chart for residual solvent testing under the revised USP <467> method.



[1] This number of analytes to be tested represents the sum of Class 1 and 2 residual solvents that can be effectively assayed using HS/GC. The actual number of analytes may be more if xylenes, ethyl benzene and *cis/trans* 1,2 dichloroethylene are differentiated, or if circumstances require the quantification of specific Class 3 residual solvents.

Analytical Reference Materials

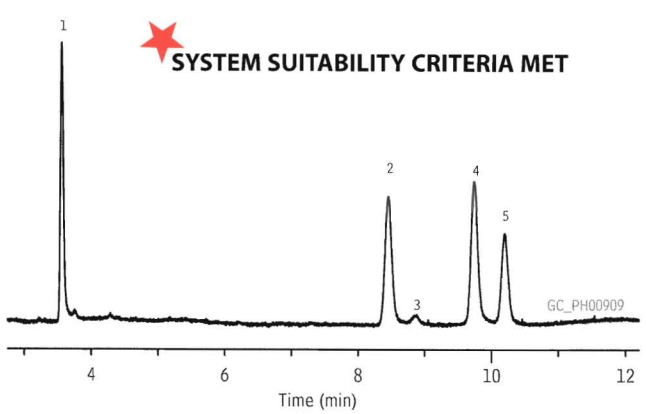
The ICH guideline classifies residual solvents by class according to toxicity. Class 1 compounds are carcinogenic and pose a risk to both the consumer and the environment. The use of these solvents must be avoided or tightly controlled. Class 2 compounds are nongenotoxic animal carcinogens and their concentration should be limited. Both Class 1 and 2 compounds require chromatographic determination and are separated into 3 test mixes: Class 1 Mixture, Class 2 Mixture A, and Class 2 Mixture B. Class 3 compounds have low toxic potential. Concentration levels of up to 0.5% are acceptable and, therefore, they can be assayed by nonspecific techniques, such as weight loss on drying. Class 2 Mixture C is not used in the second supplement of USP 30/NF 25, but contains solvents that are not readily detectable by headspace analysis. These solvents should be assayed by other appropriately validated procedures.

Procedure A - Identification

Procedure A is the first step in the identification process and is performed on a G43 column to determine if any residual solvents are present in the sample at detectable levels. First, Class 1 standard and system suitability solutions and Class 2 Mix A standard solutions are assayed under the method-specified operating conditions to establish system suitability. All peaks in the Class 1 system suitability solution must have a signal-to-noise ratio not less than 3, the Class 1 standard solution must have a 1,1,1-trichloroethane response greater than 5, and the resolution of acetonitrile and dichloromethane must be not less than 1 in the Class 2 Mixture A solution. When system suitability has been achieved, the test solutions are assayed along with the Class 1 and Class 2 Mixtures A and B standard solutions. If a peak is determined in the sample that matches a retention time and has a greater response than that of a corresponding reference material, then Procedure B is performed for verification of the analyte. In the second supplement of USP 30/NF 25, an exemption is made for 1,1,1-trichloroethane, where a response greater than 150 times the peak response denotes an amount above the percent daily exposure limit. Figures 2 through 4 (pages 3–4) illustrate the analysis of Class 1, Class 2 Mixture A, and Class 2 Mixture B residual solvent mixes by Procedure A. The resolution between acetonitrile and dichloromethane was easily achieved using an Rtx®-1301 column.

Continued on page 4.

Figure 2 USP residual solvent Class 1 standard solution on an Rtx®-1301 column (G43).

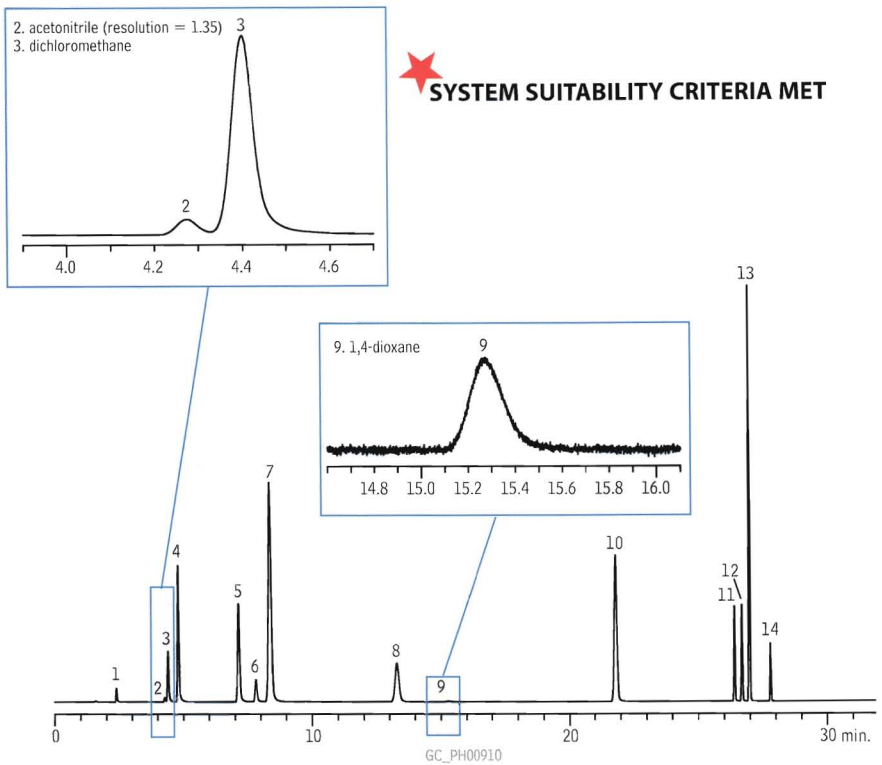


Column:	Rtx®-1301, 30m, 0.32mm ID, 1.8µm (cat.# 16092)	Headspace Conditions	
Sample:	USP <467> Class 1 standard solution (cat.# 36279) in 20mL headspace vial	Instrument:	Tekmar HT3
		Transfer line temp.:	105°C
Inj.:	headspace injection (split ratio 1:5), 1mm split liner, Siltek® deactivated (cat.# 20972-214.1)	Valve oven temp.:	105°C
		Sample temp.:	80°C
Inj. temp.:	140°C	Sample equil. time:	45 min.
Carrier gas:	helium, constant flow	Vial pressure:	10psi
Flow rate:	2.16mL/min., 35.3cm/sec.	Pressurize time:	0.5 min.
Oven temp.:	40°C for 20 min. to 240°C @ 10°C/min. (hold for 20 min.)	Loop fill pressure:	5psi
		Loop fill time:	2.00 min.
Det.:	FID @ 240°C	Inject time:	1.00 min.

Successfully Implement the Revised USP <467> Method

Continued from page 3.

Figure 3 USP residual solvent Class 2 Mixture A standard solution on an Rtx®-1301 column (G43).



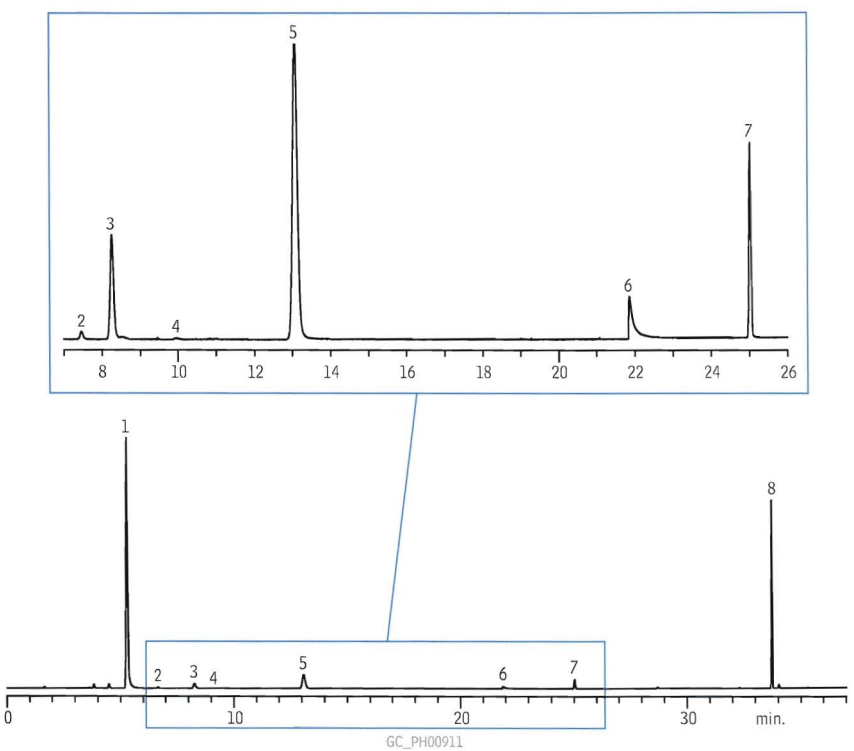
1. methanol
2. acetonitrile (resolution=1.35)
3. dichloromethane
4. *trans*-1,2-dichloroethene
5. *cis*-1,2-dichloroethene
6. tetrahydrofuran
7. cyclohexane
8. methylcyclohexane
9. 1,4-dioxane
10. toluene
11. chlorobenzene
12. ethyl benzene
13. *m*-xylene/*p*-xylene
14. *o*-xylene

Column:	Rtx®-624, 30m, 0.32 ID, 1.8µm (cat.# 10970)
Sample:	USP <467> Class 2 Mixture A standard solution (cat.# 36271) in 20mL headspace vial
Inj.:	headspace injection (split ratio 1:5), 1mm split liner, Siltek® deactivated (cat.# 20972-214.1)
Inj. temp.:	140°C
Carrier gas:	helium, constant flow
Flow rate:	2.16mL/min., 35.3cm/sec.
Oven temp.:	40°C for 20 min. to 240°C @ 10°C/min. (hold for 20 min.)
Det.:	FID @ 240°C

Headspace Conditions

Instrument:	Tekmar HT3
Transfer line temp.:	105°C
Valve oven temp.:	105°C
Sample temp.:	80°C
Sample equil. time:	45 min.
Vial pressure:	10psi
Pressurize time:	0.5 min.
Loop fill pressure:	5psi
Loop fill time:	2.00 min.
Inject time:	1.00 min.

Figure 4 USP residual solvent Class 2 Mixture B standard solution on an Rtx®-1301 column (G43).



1. hexane
2. nitromethane
3. chloroform
4. 1,2-dimethoxyethane
5. trichloroethylene
6. pyridine
7. 2-hexanone
8. tetralin

Column:	Rtx®-624, 30m, 0.32mm ID, 1.8µm (cat.# 10970)
Sample:	USP <467> Class 2 Mixture B standard solution (cat.# 36280) in 20mL headspace vial
Inj.:	headspace injection (split ratio 1:5), 1mm split liner Siltek® deactivated (cat.# 20972-214.1)
Inj. temp.:	140°C
Carrier gas:	helium, constant flow
Flow rate:	2.16mL/min., 35.3cm/sec.
Oven temp.:	40°C for 20 min. to 240°C @ 10°C/min. (hold for 20 min.)
Det.:	FID @ 240°C

Headspace Conditions

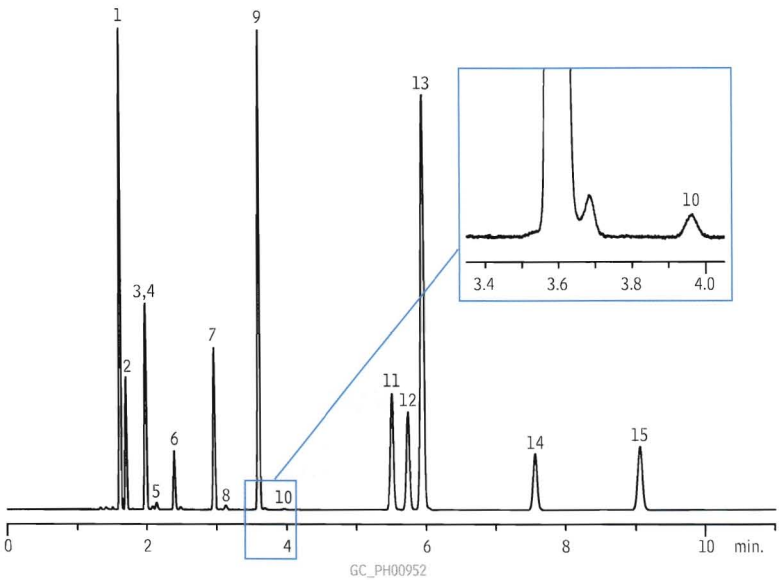
Instrument:	Tekmar HT3
Transfer line temp.:	105°C
Valve oven temp.:	105°C
Sample temp.:	80°C
Sample equil. time:	45 min.
Vial pressure:	10psi
Pressurize time:	0.5 min.
Loop fill pressure:	5psi
Loop fill time:	2.00 min.
Inject time:	1.00 min.

Successfully Implement the Revised USP <467> Method

Continued from page 5.

Figure 6 USP residual solvent Class 2 Mixture A standard solution on a Stabilwax® column (G16).

SYSTEM SUITABILITY CRITERIA MET—
RESOLUTION BETWEEN PEAKS 7 & 8 > 1.0



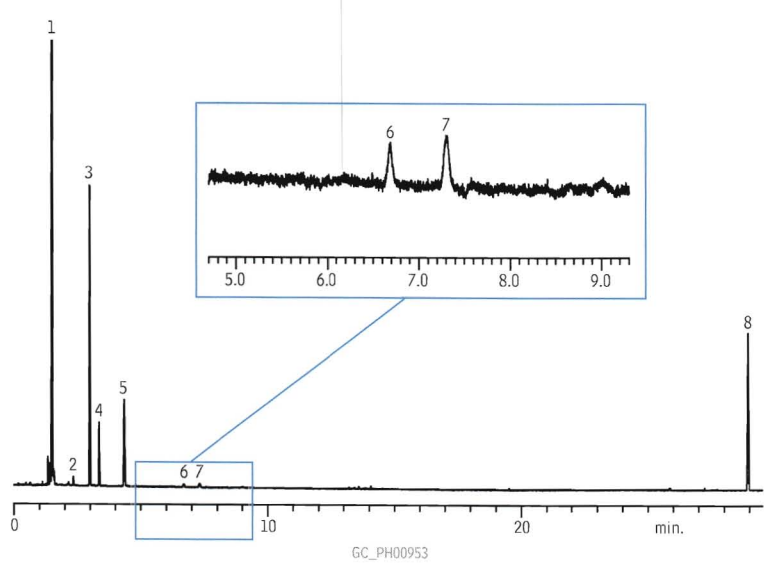
1. cyclohexane
2. methylcyclohexane
3. *trans*-1,2-dichloroethene
4. tetrahydrofuran
5. methanol
6. dichloromethane
7. *cis*-1,2-dichloroethene
8. acetonitrile
9. toluene
10. 1,4-dioxane
11. ethyl benzene
12. *p*-xylene
13. *m*-xylene
14. *o*-xylene
15. chlorobenzene

Column:	Stabilwax®, 30m, 0.32mm ID, 0.25µm (cat.# 10624)
Sample:	USP Stock Standard Residual Solvents Class 2 Mix A (cat.# 36271) in 20mL headspace vial (cat.# 24685), water diluent
Inj.:	headspace injection (split ratio 1:5), 2mm splitless liner IP deactivated (cat.# 20712)
Inj. temp.:	140°C
Carrier gas:	helium, constant flow
Flow rate:	2.15mL/min., 35.2cm/sec.
Oven temp.:	50°C for 20 min. to 165°C @ 6°C/min. (hold for 20 min.)
Det.:	FID @ 250°C

Headspace Conditions

Instrument:	Overbrook Scientific HT200H
Syringe temp.:	100°C
Sample temp.:	80°C
Sample equil. time.:	45 min.
Injection vol.:	1.0mL
Injection speed:	setting 8
Injection dwell:	5 sec.

Figure 7 USP residual solvent Class 2 Mixture B standard solution on a Stabilwax® column (G16).



1. hexane
2. 1,2-dimethoxyethane
3. trichloroethylene
4. chloroform
5. 2-hexanone
6. nitromethane
7. pyridine
8. tetralin

Column:	Stabilwax®, 30m, 0.32mm ID, 0.25µm (cat.# 10624)
Sample:	USP Stock Standard Residual Solvents Class 2 Mix B (cat.# 36272) in 20mL headspace vial (cat.# 24685), water diluent
Inj.:	headspace injection (split ratio 1:5), 2mm splitless liner IP deactivated (cat.# 20712)
Inj. temp.:	140°C
Carrier gas:	helium, constant flow
Flow rate:	2.15mL/min., 35.2cm/sec.
Oven temp.:	50°C for 20 min. to 165°C @ 6°C/min. (hold for 20 min.)
Det.:	FID @ 250°C

Headspace Conditions

Instrument:	Overbrook Scientific HT200H
Syringe temp.:	100°C
Sample temp.:	80°C
Sample equil. time.:	45 min.
Injection vol.:	1.0mL
Injection speed:	setting 8
Injection dwell:	5 sec.

Procedure B - Confirmation

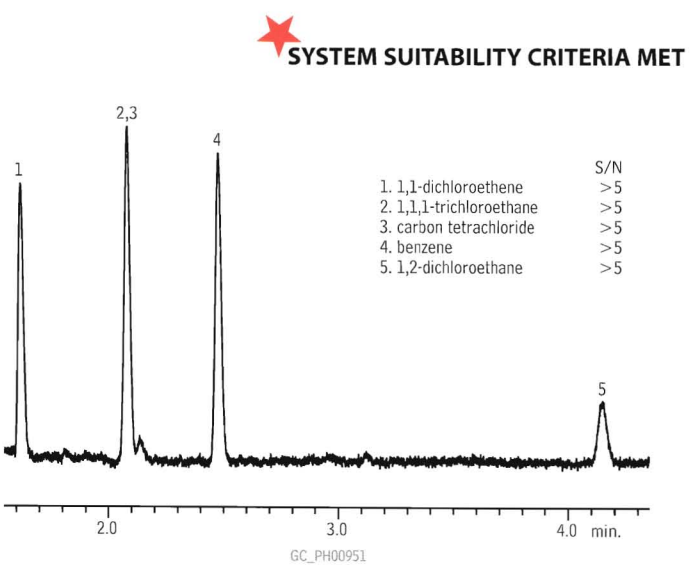
Once a residual solvent is identified and found to be above the percent daily exposure limit, Procedure B is performed to confirm analyte identity. A G16 capillary column is used here as a confirmation column, because it yields an alternate selectivity compared to a G43 column. The same standard and system suitability preparations are used in Procedures A and B. The system suitability requirements differ here in that the Class 1 standard solution must have a benzene response greater than 5 and the resolution of acetonitrile and cis-dichloroethene must not be less than 1 in the Class 2 Mixture A solution, a change from the original version. If the analyte identified in Procedure A again matches the retention time and exceeds the peak response of the reference materials (with the same exception to 1,1,1-trichloroethane), the analyst must quantify the analyte using Procedure C. Figures 5 through 7 (pages 5–6) illustrate the analysis of Class 1, Class 2 Mixture A, and Class 2 Mixture B residual solvent mixes on a Stabilwax® column. Again, the system suitability requirements were easily met.

Procedure C – Quantification

Once a residual solvent has been identified and verified, Procedure C is used to quantify the analyte by analyzing the sample against compound-specific reference materials. Individual standards are prepared by diluting the analyte in solution to a concentration of 1/20 of the concentration limit given under concentration limit Table 1 or 2 of the method. Following the procedure and instrument conditions in either Procedure A or B (whichever provides the most definitive results), a quantifiable result is produced. For water-insoluble articles, the same procedure is followed, except dimethylformamide or dimethylsulfoxide is used as the diluent.

Continued on page 6.

Figure 5 USP residual solvent Class 1 standard solution on a Stabilwax® column (G16).



Column:	Stabilwax®, 30m, 0.32mm ID, 0.25µm (cat.# 10624)	Headspace Conditions	
Sample:	USP Stock Mixture USP<467> Residual Solvents Class 1 Mix (cat.# 36279) in 20mL headspace vial (cat.# 24685), water diluent	Instrument:	Overbrook Scientific HT200H
		Syringe temp.:	100°C
		Sample temp.:	80°C
		Sample equil. time.:	45 min.
		Injection vol.:	1.0mL
Inj.:	headspace injection (split ratio 1:5), 2mm splitless liner IP deactivated (cat.# 20712)	Injection speed:	setting 8
		Injection dwell:	5 sec.
Inj. temp.:	140°C		
Carrier gas:	helium, constant flow		
Flow rate:	2.15mL/min., 35.2cm/sec.		
Oven temp.:	50°C for 20 min. to 165°C @ 6°C/min. (hold for 20 min.)		
Det.:	FID @ 250°C		

Product Listing

Capillary Column—Procedure A

Rtx®-1301 (G43) Columns (fused silica)

(Crossbond® 6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	1.80	-20 to 240°C	30-Meter	16092
0.53mm	3.00	-20 to 240°C	30-Meter	16085

Capillary Column—Procedure B

Stabilwax® Columns (fused silica)

(Crossbond® Carbowax® polyethylene glycol)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.25	40 to 250°C	30-Meter	10624
0.53mm	0.25	40 to 250°C	30-Meter	10625

Protect Your Data and Analytical Column with a

Restek Electronic Leak Detector

- Optimized sample flow path.
- A sleek, new ergonomic, hand-held design.
- Rugged side grips for added durability.
- Handy probe storage for cleanliness.
- Longer lasting battery, up to 6 hours of continuous use.
- Automatic shut-off capabilities.
- A convenient carrying and storage case.
- A universal charger set (US, European, UK and Australian plugs included).

Detect small leaks before they become a big problem.

Did you ever have a small leak turn into a costly repair? Protect your data and analytical column by using a Restek Leak Detector. Backed by a 1 year warranty, the new Restek Leak Detector will again set an industry standard for performance and affordability in hand-held Leak Detectors.

Table I Leak Detector Facts

Detectable gases:	helium, nitrogen, argon, carbon dioxide, hydrogen
Battery:	rechargeable Ni-MH internal battery pack (6 hours normal operation)
Operating Temp. Range:	32°-120°F (0°-48°C)
Humidity Range:	0-97%
Warranty:	one year
Certifications:	CE, Japan
Compliance:	WEEE, RoHS

Table II Limits of Detection

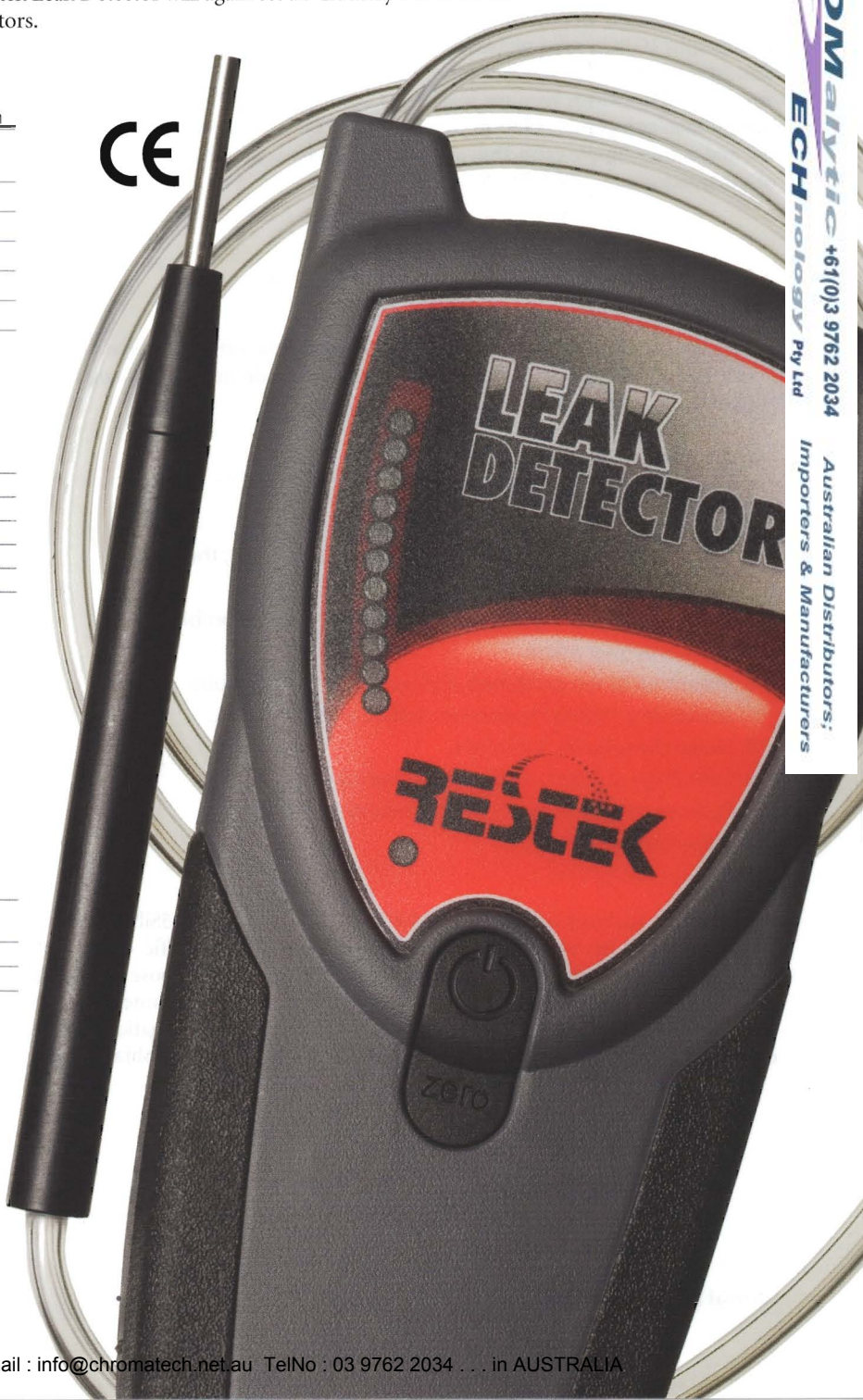
Gas	Minimum Detectable Leak Rate (atm cc/sec.)	Indicating LED Light Color
Helium	1.0×10^{-5}	Red
Hydrogen*	1.0×10^{-5}	Red
Nitrogen	1.4×10^{-3}	Yellow
Argon	1.0×10^{-4}	Yellow
Carbon Dioxide	1.0×10^{-4}	Yellow

Carrying/storage case included with purchase of unit.

Description	qty.	cat.#
Leak Detector with Universal Charger Set (US, UK, European, Australian)	ea.	22839
Soft-Side Carry/Storage Case	ea.	22657
Small Probe Adaptor	ea.	22658

Avoid using liquid leak detectors on a capillary system! Liquids can be drawn into the system.

† Caution: The Restek Electronic Leak Detector is NOT designed for determining leaks in a combustible environment. A combustible gas detector should be used for determining combustible gas leaks under any condition. The Restek Electronic Leak Detector may be used for determining trace amounts of hydrogen in a GC environment only.



Global RESTEK Advantage

Simplify and Speed Up Sample Preparation With Resprep dSPE tubes!

Here we show the extraction and clean-up of pesticide residues from olive oil samples—twice as fast as GPC, with only a fraction of the solvent required for conventional SPE.

Olive oil is considered a healthy fat source and is a staple in many recommended diets. However, concerns about potentially negative health effects associated with pesticide residues have increased consumer interest in testing. While organophosporus pesticides are currently used in olive orchards to control pests, organochlorine pesticides are still tested for as persistent organic pollutants (residues), even though they are no longer in commercial use. There are several existing methods for measuring pesticide residues in olive oil, all of which involve sample extraction and clean-up.[1] The common goal of these methods is to remove lipids that are harmful to the analytical system.[2] Efficient sample clean-up procedures are critical to maximizing sample throughput and minimizing labor and material costs. Here we demonstrate the efficiency of a dSPE clean-up procedure, as well as the capabilities of both method-specific and general purpose analytical columns.

Simple Procedure Uses Half the Time and Minimal Solvent

Sample extraction and clean-up can be accomplished with gel permeation chromatography (GPC), solid phase extraction (SPE), or dispersive solid phase extraction (dSPE) methods. However the dSPE method shown here is much less expensive than GPC (which requires specialized equipment) and uses substantially less solvent than comparable GPC or SPE methods (Table I).[3] The method is simple to use and allows sample extraction and clean-up to be accomplished in half the time of other techniques (Table II).

Extraction and dSPE Clean-up for Pesticide Residues in Olive Oil

Test sample: A 1.5mL sample of commercially obtained virgin olive oil was spiked with a standard organochlorine pesticide mix. The spiked sample was processed as follows.

1. Dilute with 1.5mL hexane.
2. Add 6mL of acetonitrile (ACN).
3. Mix for 30 minutes on a shaker.
4. Allow layers to separate (approximately 20 minutes), then collect the top (ACN) layer.
5. Repeat the liquid-liquid extraction (steps 2-4) and combine both ACN extract layers.
6. Place 1mL of the combined ACN extract in a 1.5mL tube containing 150mg magnesium sulfate and 50mg PSA.
7. Shake the tube for 2 minutes.
8. Centrifuge at 3,000 U/min. for approximately 5 minutes.
9. Remove the top layer and inject directly into the gas chromatograph system.

Extracts were analyzed using both Rtx®-CLPesticides2 and Rxi®-5Sil MS columns (Figure 1). The Rtx®-CLPesticides2 column is a method specific column that resolves all compounds. The Rxi®-5Sil MS column is a general purpose column that has one coelution that can easily be extracted by a mass spectrometer detector (MSD). Only α-BHC was not detected, a subject of further investigation, however either column can be used effectively. Recoveries of 70%-80% were obtained, levels

Table I Resprep dSPE method uses 42% and 89% less solvent than SPE and GPC methods respectively.

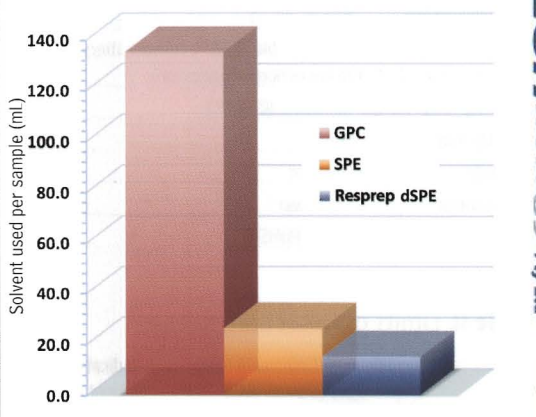


Table II Cut extraction/clean-up time by 50% using Resprep dSPE method.

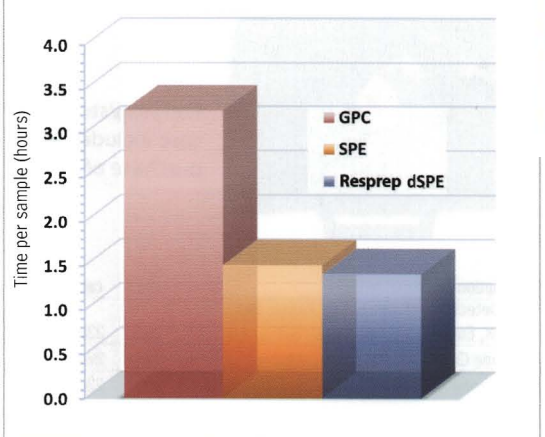
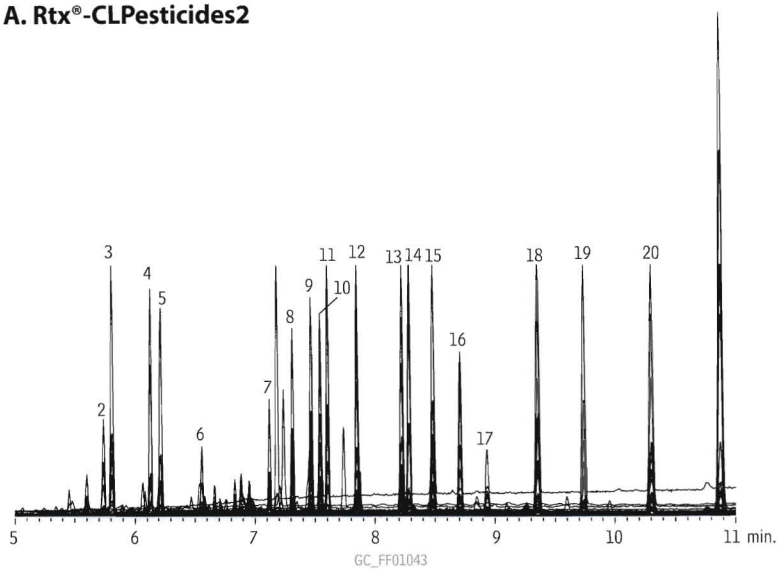
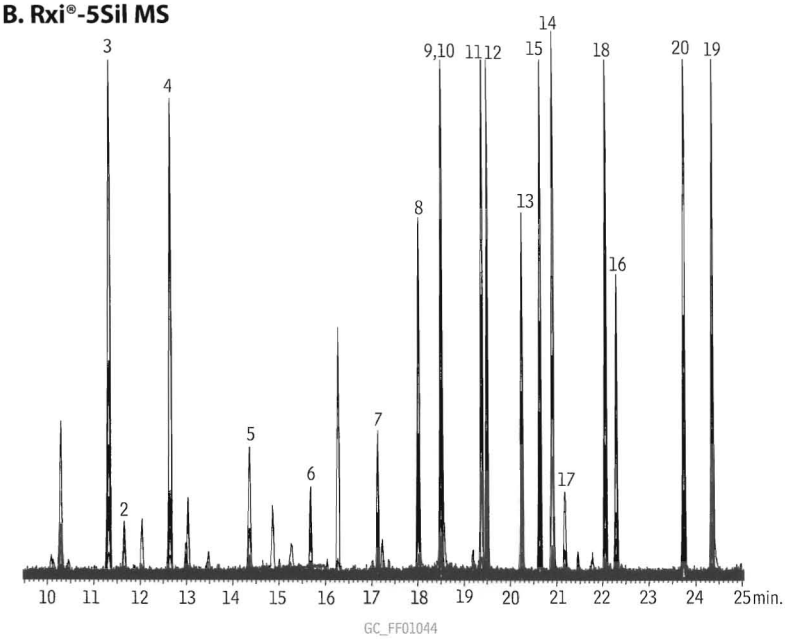


Figure 1 Chlorinated pesticide residues in olive oil are easily separated on either Rtx®-CLPesticides 2 or Rxi®-5Sil MS columns.

A. Rtx®-CLPesticides2



B. Rxi®-5Sil MS



Column:	Rtx®-CLPesticides2, 30m, 0.25mm ID, 0.20µm (cat.# 11323)
Sample:	10µg/mL Organochlorine Pesticide Mix AB # 3 (cat.# 32415) in olive oil
Inj.:	1µL, splitless (hold 0.5 min.), 3.5mm single gooseneck liner (cat.# 20962) packed with wool
Inj. temp.:	225°C
Carrier gas:	helium, constant flow
Flow rate:	1mL/min.
Oven temp.:	140°C (hold 0.5 min.) to 268°C @ 20°C/min. to 290°C @ 3°C/min. to 330°C (hold 5 min.) @ 20°C/min.
Det:	MS
Transfer line temp.:	320°C
Ionization:	EI
Mode:	SIM

Compound	Quant. ion	Q1	Q3
*1. α-BHC	219	181	109
2. γ-BHC	219	181	109
3. β-BHC	219	181	109
4. δ-BHC	219	181	109
5. heptachlor	272	237	100
6. aldrin	263	293	220
7. heptachlor epoxide	263	237	81
8. γ-chlordane	272	237	65
9. α-chlordane	272	237	65
10. endosulfan I	195	207	241
11. 4,4'-DDE	246	318	176
12. dieldrin	79	263	277
13. endrin	263	281	81
14. 4,4'-DDD	235	165	199
15. endosulfan II	195	207	-
16. 4,4'-DDT	235	165	199
17. endrin aldehyde	67	250	345
18. endosulfan sulfate	272	229	239
19. methoxychlor	227	274	-
20. endrin ketone	67	317	281
* not present			

comparable to conventional SPE—without the necessity of vacuum manifolds or high pressure systems. The GPC method attained recoveries of > 95%. However this method requires large amounts of solvent and takes over twice as long as other methods.

The dSPE method shown here is an efficient, cost-effective way to clean up chlorinated pesticide residues in olive oil. With good recoveries and minimal matrix interference, it is an easy way to reduce solvent usage, compared to conventional SPE, and is more cost-effective than GPC.

References
1. C. Lentza-Rizos, E.J. Avramides, Rev. Environ. Contam. Toxicol. 141 (1995) 111.
2. S. Cunha, S. Lehotay, K. Mastovska, J. Sep. Sci. 30 (2007) 620.
3. M. Crawford, M. Halvorson, J. Stevens, The Examination and Automation of GPC, SPE and QuEChERS Utilized in Extracting Pesticides from Olive Oil. HPLC 2008 poster presentation.

Product Listing

dSPE Tube for Clean-Up of Pesticide Residue Samples

Description	Material	Methods	qty.	cat#
2mL Micro-Centrifuge Tubes for dSPE				
Resprep Q250	150mg $MgSO_4$, 50mg PSA	AOAC 2007.1	100-pk.	26124

PSA—primary and secondary amine exchange material.

Organochlorine Pesticide Mix AB # 3

(20 components)

aldrin	dieldrin
α-BHC	endosulfan I
β-BHC	endosulfan II
δ-BHC	endosulfan sulfate
γ-BHC (lindane)	endrin
α-chlordane	endrin aldehyde
γ-chlordane	endrin ketone
4,4'-DDD	heptachlor
4,4'-DDE	heptachlor epoxide (isomer B)
4,4'-DDT	methoxychlor

2,000µg/mL each in hexane:toluene (1:1), 1mL/ampul
cat. # 32415 (ea.)

Rtx®-CLPesticides2 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.20	-60 to 320/340°C	30-Meter	11323

Rxi®-5Sil MS Columns (fused silica)

(Crossbond®, selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	13623

For more SPE products, please visit us at **www.restek.com** or contact your local Restek representative.

Beyond C18—Increase Retention of Hydrophilic Compounds Using Biphenyl Columns

*Searching for a better way to retain hydrophilic aromatic drug compounds? Biphenyl phases, such as the **Pinnacle™ DB Biphenyl** column, provide greater retention than alkyl phases. Use a Biphenyl column to separate difficult-to-retain polar aromatics from unretained matrix contaminants.*

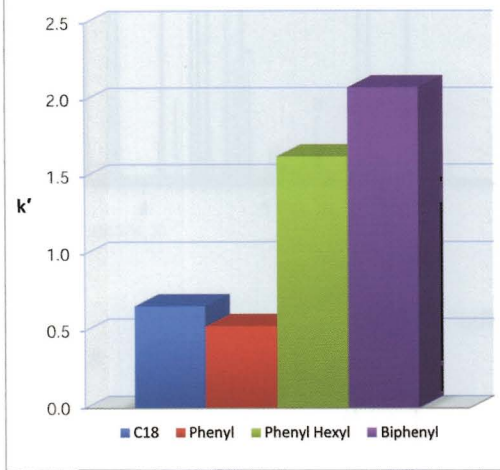
Many drug classes include compounds with aromatic ring structures, some of which also contain a sulfone or sulfoxide group. Both sulfur groups have dipole moments, adding a hydrophilic character to compounds containing these functional groups. The analysis of hydrophilic compounds on a traditional alkyl column (e.g., C18) can be problematic, since alkyl columns depend on hydrophobic (dispersive) interactions for retention. Since the sulfone and sulfoxide groups contain π bonds, the Biphenyl column's affinity toward compounds containing these bonds makes it a logical choice when increased retention of compounds containing these groups is desired.

To explore the selectivity of the biphenyl phase towards sulfur-containing aromatic compounds, phenyl sulfone, a simple probe, was analyzed on alkyl (C18), phenyl, phenyl hexyl, and Biphenyl columns to determine the relative retention of each phase, as measured by capacity factor (k'). In order to ensure separation of analytes from unretained contaminants, a minimum k' value of 2 is recommended for most analyses, however in cases where there is little to no matrix interference, a k' of 1 may be acceptable. The data in Figure 1 show that phenyl sulfone is retained to a much greater degree on the Pinnacle™ DB Biphenyl column, than on the other phases tested (k' = 2.08). This is due to the unique retention mechanism of the biphenyl stationary phase, which can interact with both the hydrophobic aromatic ring and the hydrophilic sulfone group through π-π interactions. Although the phenyl stationary phase also allows for the use of π-π interactions, the biphenyl phase has a larger electron cloud and is significantly more retentive.

To further test the retention of the Biphenyl column, a second set of probes, consisting of compounds in the NSAID family, was analyzed. Tenoxicam, which contains a sulfone group, and sulfinpyrazone, which contains a sulfoxide group, were analyzed along with a void marker (uracil). Although these compounds are more complex than the probe used in the first experiment, the same pattern of retention was observed (Figure 2). The Pinnacle™ DB Biphenyl column exhibited the greatest retention for tenoxicam. With k' values of 0.33 on the C18 and 0.49 on the phenyl columns, tenoxicam shows almost no retention on these stationary phases. The phenyl hexyl phase performed slightly better with a k' value of 1.52 for tenoxicam. However, when tenoxicam was analyzed on the Biphenyl column under the same conditions, the k' value increased to 2.22, a value much more likely to provide adequate resolution from matrix components. Sulfinpyrazone, a less polar compound, also followed the same pattern of retention (Table I).

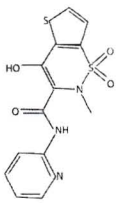
The improved retention for hydrophilic aromatics shown here is due to the unique π-π interaction retention mechanism of the Biphenyl phase. This mechanism is particularly useful for analysis of sulfone- and sulfoxide-containing drug compounds, which are not easily retained on alkyl or phenyl phases. The Biphenyl phase provides greater retention than alkyl and phenyl phases and is ideal for separating difficult-to-retain polar aromatics from unretained matrix contaminants.

Figure 1 The Biphenyl phase is more retentive for phenyl sulfone than other alkyl and phenyl phases.

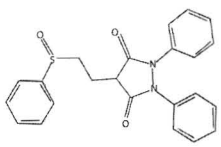


Biphenyl columns are much more effective than alkyl, phenyl, or phenyl hexyl phases when increased retention of hydrophilic aromatics is desired.

Figure 2 Only the Biphenyl phase retains both test probes to k' > 2, the level recommended to ensure separation from unretained matrix contaminants.



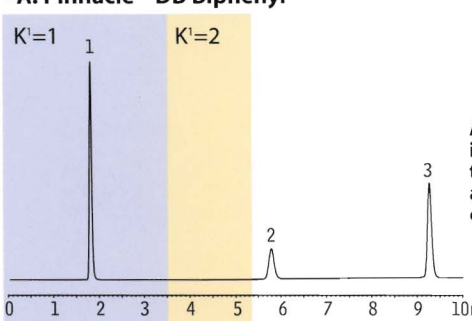
2. tenoxicam



3. sulfinpyrazone

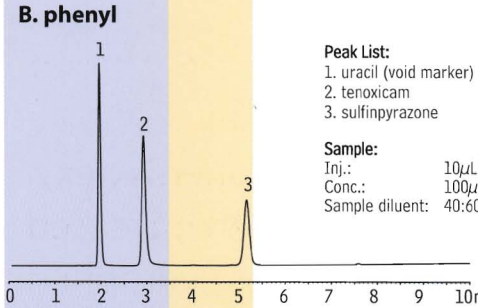
More retention with < ½ the carbon load, compared to phenyl hexyl columns

A. Pinnacle™ DB Biphenyl



A minimum k' value of 2 is generally recommended to fully separate target analytes from matrix contaminants.

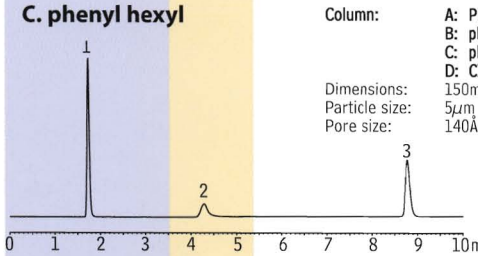
B. phenyl



Peak List:
1. uracil (void marker)
2. tenoxicam
3. sulfinpyrazone

Sample:
Inj.: 10µL
Conc.: 100µg/mL each component
Sample diluent: 40:60 water:0.1% formic acid:methanol

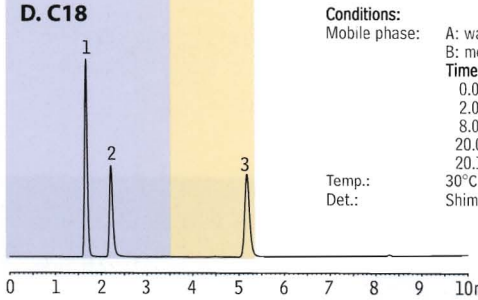
C. phenyl hexyl



Column: A: Pinnacle™ DB Biphenyl (cat.# 9409565)
B: phenyl
C: phenyl hexyl
D: C18

Dimensions: 150mm x 4.6mm
Particle size: 5µm
Pore size: 140Å

D. C18



Conditions:
Mobile phase: A: water w/ 0.1% formic acid
B: methanol

Time (min.)	Flow (mL/min.)	%B
0.0	1.0	60
2.0	1.0	60
8.0	1.0	90
20.0	1.0	90
20.1	1.0	60

Temp.: 30°C
Det.: Shimadzu PDA (SPD-M20A) @ 254nm

Table I Biphenyl columns show improved retention of sulfone- and sulfoxide-containing aromatic drugs.

K' Value	Biphenyl	Phenyl hexyl	Phenyl	C18
Tenoxicam	2.23	1.39	0.637	0.235
Sulfinpyrazone	4.18	3.90	1.88	1.89

Product Listing

Pinnacle™ DB Biphenyl Columns (USP L11)

particle size: 1.9µm, 3µm or 5µm, spherical
pore size: 140Å
carbon load: 8%
endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

3µm Column, 1.0mm	cat. #
30mm	9409331
50mm	9409351
100mm	9409311
150mm	9409361
3µm Column, 2.1mm	**cat. #**
30mm	9409332
50mm	9409352
100mm	9409312
150mm	9409362
3µm Column, 3.2mm	**cat. #**
30mm	9409333
50mm	9409353
100mm	9409313
150mm	9409363
3µm Column, 4.6mm	**cat. #**
30mm	9409335
50mm	9409355
100mm	9409315
150mm	9409365
5µm Column, 1.0mm	**cat. #**
30mm	9409531
50mm	9409551
100mm	9409511
150mm	9409561
200mm	9409521
250mm	9409571
5µm Column, 2.1mm	**cat. #**
30mm	9409532
50mm	9409552
100mm	9409512
150mm	9409562
200mm	9409522
250mm	9409572
5µm Column, 3.2mm	**cat. #**
30mm	9409533
50mm	9409553
100mm	9409513
150mm	9409563
200mm	9409523
250mm	9409573
5µm Column, 4.6mm	**cat. #**
30mm	9409535
50mm	9409555
100mm	9409515
150mm	9409565
200mm	9409525
250mm	9409575

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