Environmental HPLC

Applications • Columns • Reference Materials

RESTEK

Turning Visions into Reality™

Environmental HPLC

A number of environmental analyses are well-suited to HPLC. Compatibility with thermally labile compounds, aqueous samples, and larger injection volumes, relative to GC, makes HPLC an attractive option. Detection for environmental compounds is most frequently by UV/Vis, with fluorescence detection used for specific applications and LC/MS increasingly finding its way into the environmental lab.

Restek has a suite of columns to support a range of environmental HPLC applications. Additionally, we offer analytical reference materials and solid phase extraction products for many of these methods.

Carbamates

Carbamates are widely used insecticides that pose a health risk as endocrine disruptors. Our Ultra Carbamate column, in a 50mm length, separates the common carbamates listed in US EPA Method 531 in less than 10 minutes (Figure 1), significantly less than the time required by traditional C18 columns. When using the short column with fluorescence detection, the total dead volume for the system, including the internal volume of the post-column reactor, must be less than 650µL; for older post-column reactor systems, we recommend a 250 x 4.6mm column.

In addition to the best column choice for the analysis, we offer reference mixes for Method 531 carbamates (see page 8), a performance check mix, and the specified internal standard, 4-bromo-3,5-dimethylphenyl-N-methylcarbamate (BDMC).

Polyaromatic Hydrocarbons (PAHs)

PAHs are suspected of being mutagenic and/or carcinogenic. Most HPLC methods for PAHs recommend using a C18 column with fluorescence and/or UV/Vis detection; we have found that an optimized C18 phase gives the best overall resolution. Our Pinnacle™ II PAH columns have a highly reproducible modified alkyl phase, specifically developed for this application. Figure 2 shows the analysis of 16 target PAHs in less than 18 minutes, and Figure 3 shows a separation of 20 target PAHs and related compounds, in less than 6 minutes, using a 5cm column.

In addition to rugged, reproducible Pinnacle™ II PAH columns, we offer analytical reference mixtures of the PAHs specified in US EPA methodology (see page 8 and our catalog or website). Choose the concentrations and solvent system that best meet your needs, or ask us about custom-prepared formulations.

Figure 1 Fast, efficient separation of carbamates on an Ultra Carbamate column.

Peak List:
1. aldicarb sulfone
2. aldicarb sulfoxide
3. oxamyl
4. methomyl
5. 3-hydroxycarbofuran
6. aldicarb
7. propoxur
8. carbofuran
9. carbaryl
10. methiocarb
11. 4-bromo-3,5-dimethylcarbamate

Sample:
Inj.: 5µL cat. # 32274 and cat. # 32273 mixed 50:50
Conc.: 50µg/mL each
Solvent: methanol

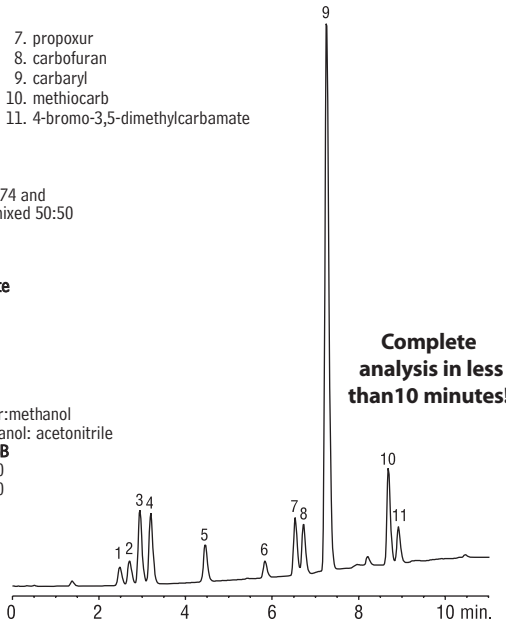
Column: **Ultra Carbamate**
Cat. #: 9177355
Dimensions: 50 x 4.6mm
Particle size: 3µm
Pore size: 100Å

Conditions:
Mobile phase: A: 90:10 water:methanol
B: 90:10 methanol: acetonitrile

Time (min.)	%B
0	10
10	90

Flow: 1.5mL/min.
Temp.: 27°C
Det.: UV @ 220nm

Complete analysis in less than10 minutes!



LC_0225

Figure 2 Baseline separation of 16 PAHs in less than 18 minutes on a Pinnacle™ II PAH column.

Sample:
Inj.: 5µL 610 PAH Calibration Mix A (Cat.# 31264)
Solvent: 1:9 methylene chloride:acetonitrile

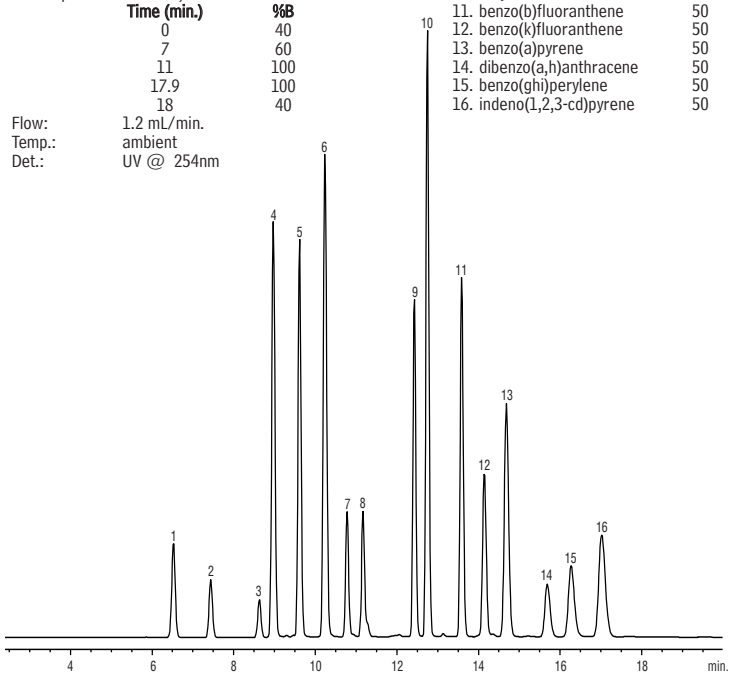
Column: **Pinnacle™ II PAH**
Cat. #: 9219563
Dimensions: 150 x 3.2mm
Particle size: 5µm
Pore size: 110Å

Conditions:
Mobile phase: A: water, B: acetonitrile

Time (min.)	%B
0	40
7	60
11	100
17.9	100
18	40

Flow: 1.2 mL/min.
Temp.: ambient
Det.: UV @ 254nm

Peak List:	Conc. (µg/mL)
1. naphthalene	100
2. acenaphthylene	100
3. acenaphthene	100
4. fluorene	100
5. phenanthrene	50
6. anthracene	100
7. fluoranthene	50
8. pyrene	50
9. benzo(a)anthracene	50
10. chrysene	50
11. benzo(b)fluoranthene	50
12. benzo(k)fluoranthene	50
13. benzo(a)pyrene	50
14. dibenzo(a,h)anthracene	50
15. benzo(ghi)perylene	50
16. indeno(1,2,3-cd)pyrene	50



LC_0303

Figure 3 Fast, efficient separation of 20 target PAHs and related compounds, using a 5cm Pinnacle™ II PAH column.

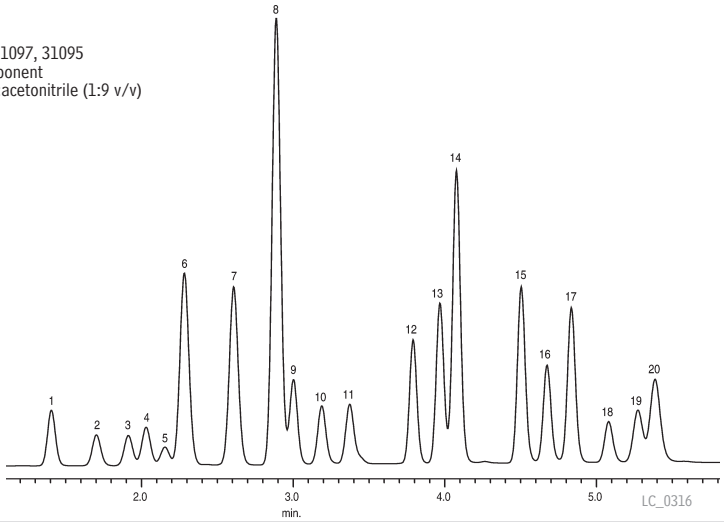
Sample:
Inj.: 5µL cat.# 31843, 31097, 31095
Conc.: 5-10ppm each component
Sample diluent: methylene chloride:acetonitrile (1:9 v/v)

Column: **Pinnacle™ II PAH**
Cat. #: 9219555
Dimensions: 50 x 4.6mm
Particle size: 5µm
Pore size: 110Å

Conditions:
Mobile phase: A: water
B: acetonitrile

Time (min.)	%B
0	50
1.5	60
4.0	100
5.5	100
5.6	50

Flow: 3.0mL/min.
Temp.: ambient
Det.: UV @ 254nm



Peak List:
1. naphthalene
2. acenaphthylene
3. 1-methylnaphthalene
4. 2-methylnaphthalene
5. acenaphthene
6. fluorene
7. phenanthrene
8. anthracene
9. *o*-terphenyl
10. fluoranthene
11. pyrene
12. *p*-terphenyl
13. benzo(a)anthracene
14. chrysene
15. benzo(b)fluoranthene
16. benzo(k)fluoranthene
17. benzo(a)pyrene
18. dibenzo(a,h)anthracene
19. benzo(ghi)perylene
20. indeno(1,2,3-cd)pyrene

Explosives

Testing of residual materials is important when monitoring the disposal of expired or deteriorated munitions. US EPA Method 8330 was developed for quantifying 14 commonly monitored explosives. The method calls for reversed phase HPLC with UV detection, using a primary column (e.g., C18; see Figure 4) and a confirmation column. While cyano phases typically have been used for the confirmation column, resolution of the target explosive compounds is poor. The Pinnacle™ II Biphenyl column provides excellent resolution of Method 8330 explosives, as shown in Figure 5, and selectivity is markedly different from C18 phases, making the Pinnacle™ II Biphenyl column an ideal confirmation column. Separations on either phase are effected using an isocratic, water:methanol mobile phase. If a cyano phase must be used for confirmation, we recommend a Pinnacle™ II Cyano column. Restek analytical reference materials for Method 8330 are described on page 8.

Figure 4 An Ultra C18 column is an outstanding primary column for explosives analysis.

Sample:
Inj.: 5µL (Cat. #: 31452, 31453, 31450, 31451)
Conc.: 30µg/mL (30ppm) each
Sample diluent: acetonitrile

Column: **Ultra C18**
Cat. #: 9174575
Dimensions: 250 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: water:methanol, 44:56, v/v
Flow: 1.0mL/min.
Temp.: 30°C
Det.: UV @ 254nm

Peak List:
1. HMX
2. RDX
3. 1,2-dinitrobenzene (SS)
4. 1,3,5-trinitrobenzene
5. 1,3-dinitrobenzene
6. tetryl
7. nitrobenzene
8. 3,4-dinitrotoluene (IS)

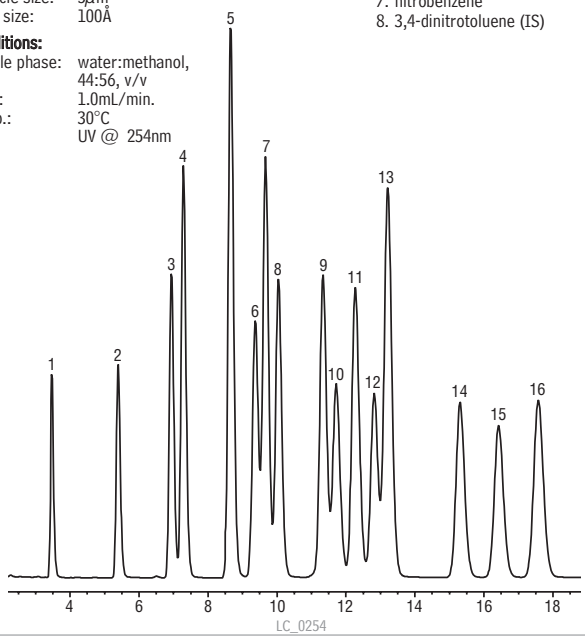


Figure 5 Excellent resolution of explosives, using a Pinnacle™ II Biphenyl confirmation column.

9. 2,4,6-trinitrotoluene
10. 4-amino-2,6-dinitrotoluene
11. 2-amino-4,6-dinitrotoluene
12. 2,6-dinitrotoluene
13. 2,4-dinitrotoluene
14. 2-nitrotoluene
15. 4-nitrotoluene
16. 3-nitrotoluene

Sample:
Inj.: 10µL (Cat. #: 31452, 31453, 31450, 31451)
Conc.: 30µg/mL (30ppm) each
Sample diluent: acetonitrile

Column: **Pinnacle™ II Biphenyl**
Cat. #: 9209565
Dimensions: 150 x 4.6mm
Particle size: 5µm
Pore size: 110Å

Conditions:
Mobile phase: water:methanol, 50:50, v/v
Flow: 1.5 mL/min.
Temp.: ambient
Det.: UV @ 254nm

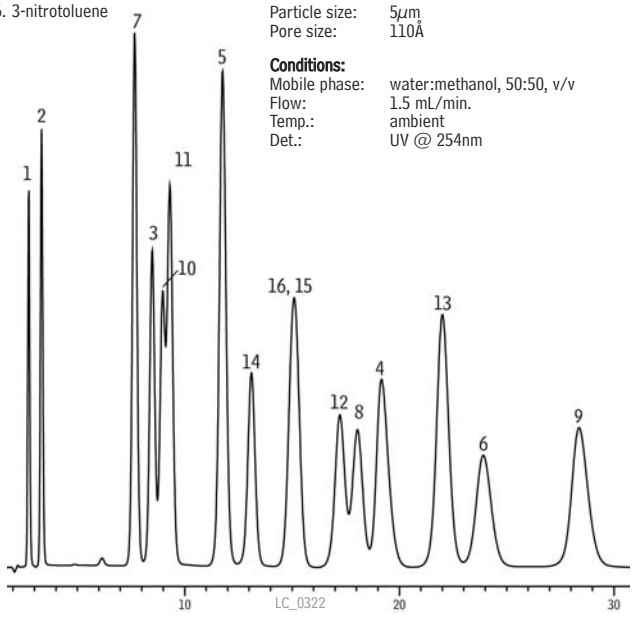
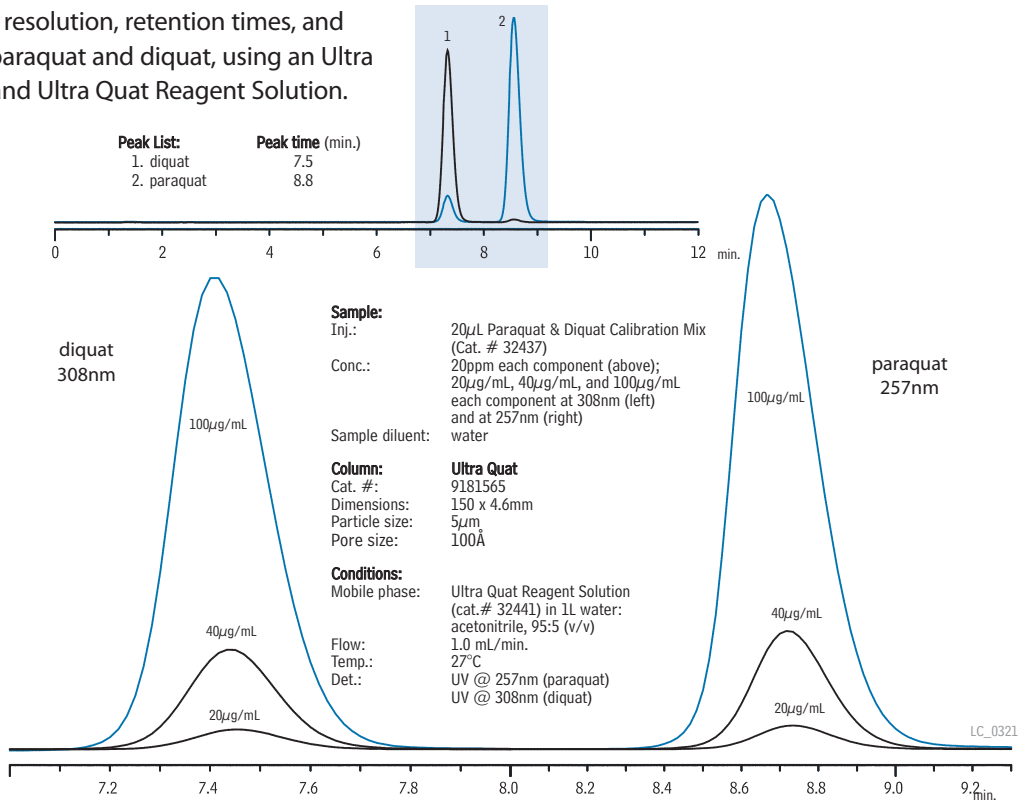


Figure 6 Consistent resolution, retention times, and peak symmetry for paraquat and diquat, using an Ultra Quat HPLC column and Ultra Quat Reagent Solution.

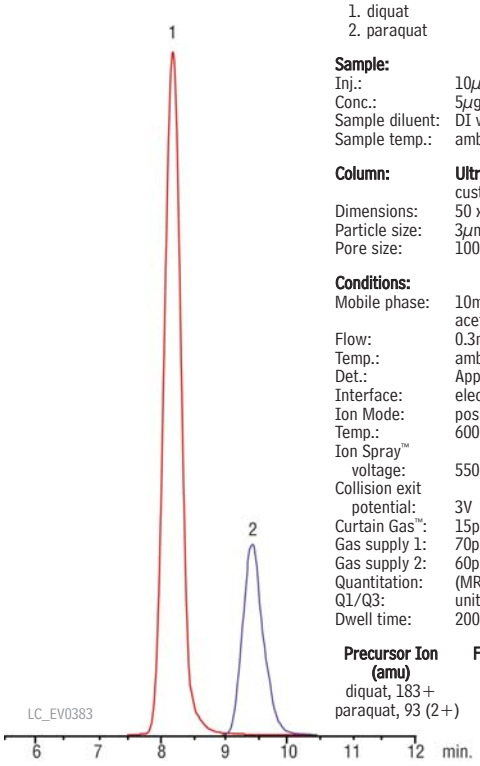


Paraquat/Diquat

Highly charged dual quaternary amines paraquat (methyl viologen) and diquat are readily soluble in water, but are difficult to retain by standard reversed phase HPLC. Ion pairing methods (as in US EPA Method 549) and specialty columns have been developed for this analysis. However, methods involving ion pairing agents inherently are more complex and less reproducible, due to the complex chemistry and methodology and to variations among manufacturers' HPLC columns.

Restek HPLC chemists have developed a simple, effective, reliable system for analyzing paraquat and diquat, based on the Ultra Quat HPLC column and a unique mobile phase: Ultra Quat Reagent Solution (cat.# 32441). The analysis can be performed on a conventional HPLC system, with UV detection. The separation relies on chaotropism: the ability of the mobile phase to alter the interactions among analyte, mobile phase, and stationary phase. Requiring only the reagent solution, water, and acetonitrile, this simple system is an excellent alternative to complex and irreproducible ion pairing methods. Further, the detection limit, 0.03ppb for either compound, is a significant improvement over current methodology.

Figure 7 Fast, sensitive LC/MS/MS analysis of paraquat & diquat using an Ultra Quat HPLC column and an API™ 3200 mass spectrometer.



Peak List
1. diquat
2. paraquat

Sample:
Inj.: 10µL
Conc.: 5µg/mL each component
Sample diluent: DI water
Sample temp.: ambient

Column: **Ultra Quat** (cat.# 5181352, custom column, available on request)
Dimensions: 50 x 2.1 mm
Particle size: 3µm
Pore size: 100Å

Conditions:
Mobile phase: 10mM heptafluorobutyric acid: acetonitrile, 95:5 (v/v)
Flow: 0.3mL/min.
Temp.: ambient
Det.: Applied Biosystems/Sciex API™3200
Interface: electrospray
Ion Mode: positive
Temp.: 600°C
Ion Spray™ voltage: 5500V
Collision exit potential: 3V
Curtain Gas™: 15psi
Gas supply 1: 70psi
Gas supply 2: 60psi
Quantitation: (MRM)
Q1/Q3: unit resolution
Dwell time: 200 ms

Precursor Ion (amu)	Fragment Ion (amu)	DP (V)	Collision Energy (eV)
diquat, 183+	157+	30	30
paraquat, 93 (2+)	171+	20	20

*Data courtesy of Houssain El Aribi, Ph.D., LC/MS Product and Application Specialist, MDS SCIEX, 71 Four Valley Drive, Concord, Ontario, Canada, L4K 4V8

Figure 6 shows analyses of paraquat and diquat reference standards at 20-100µg/mL; resolution, retention times, and peak symmetry are highly consistent. For more information about this application, please request Application Note 580006.

Most recently, LC/MS/MS detection has been used for this application, with excellent results (Figure 7). Heptafluorobutyric acid is used as the volatile mobile phase modifier. Please note that all glassware used in the preparation and analysis of paraquat and diquat must be deactivated (e.g., with DMDCS, cat.# 31840), due to interactions of these active compounds with untreated glass surfaces.

In addition to the column and reagent solution, analytical reference materials and solid phase extraction tubes for this analysis are available from Restek - see pages 7 and 8.

Carbonyls

Carbonyl compounds, including lower molecular weight aldehydes and ketones, are receiving increased attention, as short-term exposure irritates the eyes, skin, and mucous membranes. Formaldehyde is a major promoter in the formation of photochemical ozone, and is the target compound for US EPA Methods 8315 and TO-11A. At least 14 other carbonyl compounds can be detected and quantified using this methodology, which is based on the specific interaction between carbonyl compounds and 2,4-dinitrophenylhydrazine (DNPH).

An Allure™ C18 HPLC column is an excellent choice for fast, effective separations of DNPH derivatives of aldehydes and ketones (Figure 8). We also offer a 15-component DNPH-aldehyde reference standard (page 8); for convenience, our Certificate of Analysis lists concentrations for both the aldehydes/ketones and the DNPH derivatives.

Herbicides

Phenoxyacid herbicides, such as 2,4-D and Silvex (2,4,5-TP), can be found in the acid form, or as the salts or esters. Traditionally, these compounds have been analyzed by gas chromatography (e.g., by EPA Method 8151). To make them amenable to GC, however, they must be converted to the methyl esters using a toxic derivatizing agent, such as diazol. HPLC is an attractive option to this time-consuming, hazardous procedure. For example, US EPA Method 555 discusses the analysis of these herbicides, in the acid form, in

Figure 8 Fast, reliable analysis of aldehydes and ketones as DNPH derivatives on an Allure™ C18 column.

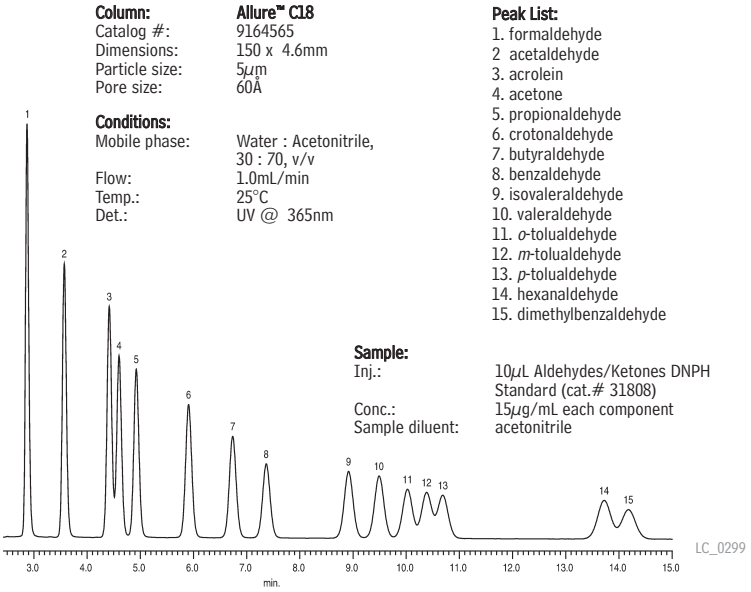
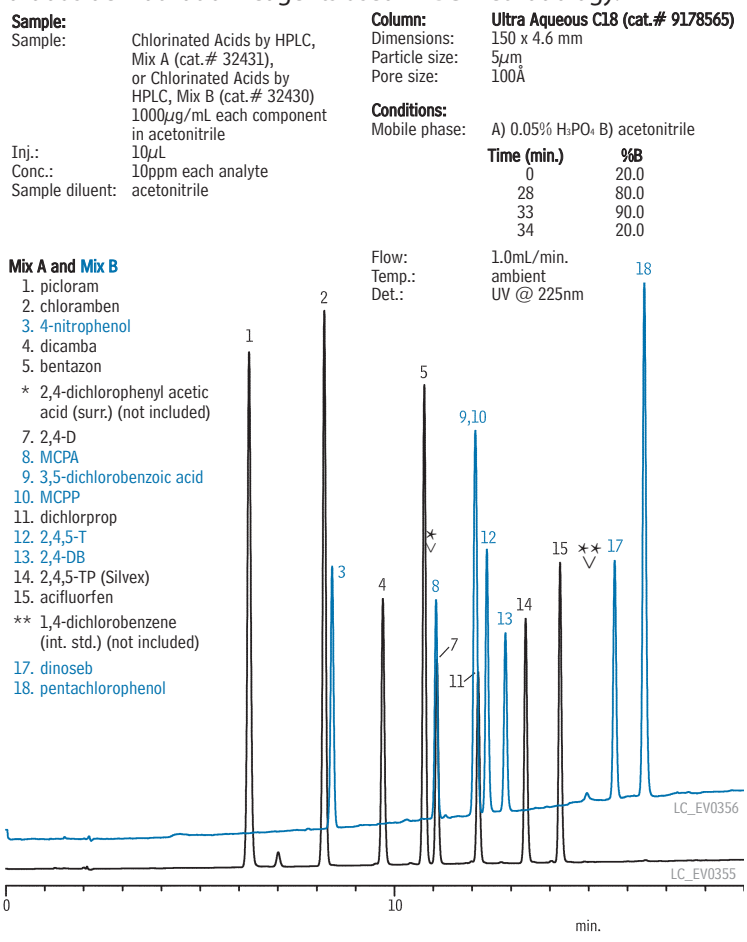


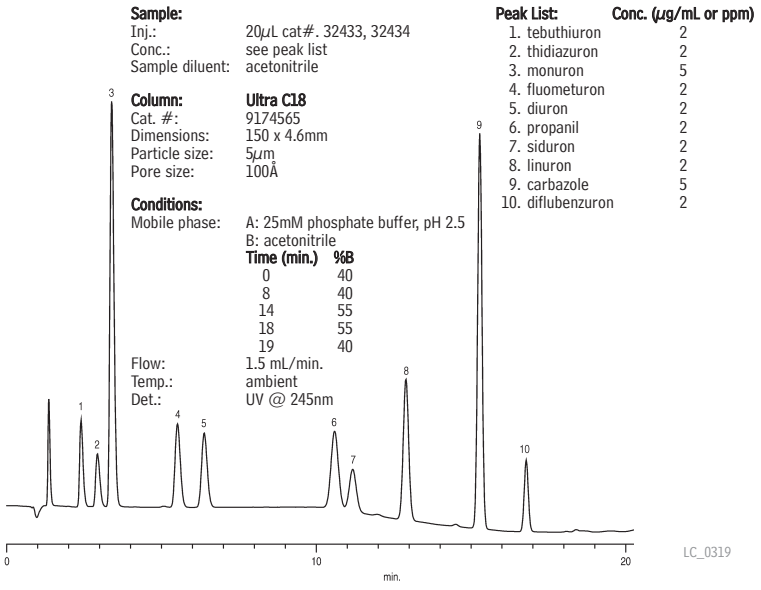
Figure 9 HPLC analysis of phenoxyacid herbicides on an Ultra Aqueous C18 column saves time and eliminates exposure to hazardous derivatization reagents used in GC methodology.



drinking water. Method 8321 is a general-purpose HPLC procedure for semivolatile compounds, including phenoxyacid herbicides. To minimize coelutions, these methods divide the herbicides into two groups. Figure 9 shows the analysis of the phenoxyacid herbicides in their acid forms on an Ultra Aqueous C18 column. A gradient procedure is useful when analyzing such a range of herbicides; for analyses of 2,4-D and Silvex, an isocratic mobile phase saves analysis and reequilibration time. An Allure™ Basix column is the recommended confirmation column.

Phenylurea herbicides listed in US EPA Method 532 can be analyzed on an Ultra C18 column (Figure 10). By lowering the pH to 2.5, using phosphate buffer, and employing a gradient mobile phase program, 10 target phenylurea herbicides can be separated efficiently. We recommend an Ultra Cyano column for confirmation.

Figure 10 Effective separation of phenyluea herbicides, using an Ultra C18 column.



Product Listing

Allure™ C18 Columns (USP L1)
Excellent Columns for LC/MS and ELSD

Physical Characteristics:

particle size: 3µm or 5µm, spherical
pore size: 60Å
carbon load: 27%
endcap: fully endcapped
pH range: 2.5 to 7.5
temperature limit: 80°C

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
5µm Columns				
150mm	9164561	9164562	9164563	9164565
250mm	9164571	9164572	9164573	9164575

Ultra Aqueous C18 Columns (USP L1)

Physical Characteristics:

particle size: 3 or 5µm, spherical
pore size: 100Å
endcap: no
pH range: 2.5 to 7.5
temperature limit: 80°C

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
5µm Columns				
150mm	9178561	9178562	9178563	9178565
250mm	9178571	9178572	9178573	9178575

Ultra C18 Columns (USP L1)

Physical Characteristics:

particle size: 3µm or 5µm, spherical
pore size: 100Å
carbon load: 20%
endcap: fully endcapped
pH range: 2.5 to 7.5
temperature limit: 80°C

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.0mm ID cat.#	4.6mm ID cat.#
5µm Columns					
30mm	9174531	9174532	9174533	—	9174535
50mm	9174551	9174552	9174553	—	9174555
100mm	9174511	9174512	9174513	9174514	9174515
150mm	9174561	9174562	9174563	9174564	9174565
200mm	9174521	9174522	9174523	—	9174525
250mm	9174571	9174572	9174573	—	9174575

for **more** info

For a complete listing of Restek HPLC columns, visit us online at **www.restek.com/hplc** or see our current chromatography products catalog.

Ultra Carbamate Columns

Physical Characteristics:

particle size: 3µm, spherical
pore size: 100Å
pH range: 2.5 to 7.5
temperature limit: 80°C

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.0mm ID cat.#	4.6mm ID cat.#
3µm Columns					
50mm	9177351	9177352	9177353	9177354	9177355
5µm Columns					
250mm	—	—	—	—	9177575

restek **innovation**!

An Ultra Carbamate column can process as many as 3 to 4 samples per hour, versus less than 2 samples per hour on a general-purpose C18 column.

Pinnacle™ II PAH Columns

Physical Characteristics:

particle size: 5µm, spherical
pore size: 110Å
endcap: fully endcapped
pH range: 2.5 to 10
temperature limit: 80°C

Length	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
5µm Columns			
50mm	9219552	9219553	9219555
150mm	—	9219563	9219565

Allure™ Basix Column (USP L10)
Excellent Column for LC/MS and ELSD

Physical Characteristics:

particle size: 3µm or 5µm, spherical
pore size: 60Å
carbon load: 12%
endcap: fully endcapped
pH range: 2.5 to 7.5
temperature limit: 80°C

Length	4.6mm ID cat.#
5µm Column	
150mm	9161565

ordering **note**

To order a 2.1mm, 3.2mm, or 4.6mm ID column with a Trident™ Integral Inlet Fitting, add "-700" to the catalog number for the column.
Example: 100mm x 4.6mm ID Ultra C18 column with Trident™ Integral Inlet Fitting: 9174315-700
Nominal additional charge

Don't forget to order the appropriate XG-XF fitting (cat. #25026 or 26062).

Ultra Quat Columns

Physical Characteristics:

particle size: 5µm, spherical
pore size: 100Å
pH range: 2.5 to 7.5
temperature limit: 80°C

Length	4.6mm ID cat.#
5µm Column	
150mm	9181565

Pinnacle™ II Biphenyl Columns

Physical Characteristics:

particle size: 5µm
pore size: 110Å
endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

Length	4.6mm ID cat.#
5µm Columns	
150mm	9209565
250mm	9209575

Pinnacle™ II Cyano Column (USP L10)

Physical Characteristics:

particle size: 3µm or 5µm, spherical
pore size: 110Å
carbon load: 4%
endcap: fully endcapped
pH range: 2.5 to 7.5
temperature limit: 80°C

Length	4.6mm ID cat.#
5µm Columns	
250mm	9216575

Ultra Cyano Column (USP L10)

Physical Characteristics:

particle size: 3µm or 5µm, spherical
pore size: 100Å
carbon load: 8%
endcap: fully endcapped
pH range: 2.5 to 7.5
temperature limit: 80°C

Length	4.6mm ID cat.#
5µm Columns	
150mm	9106565

Specialty SPE Cartridges

Specifically designed to provide consistent and reproducible results for the method stated.

Description	Applications	Tube Volume, Bed Weight	qty.	cat.#
EPA Method 549.2 (Ultra Quat SPE)	HPLC analysis of paraquat/diquat.	6mL, 500mg	30-pk.	25499
EPA Method 8321 (AH SPE)	HPLC analysis of phenoxy acid herbicides.	6mL, 500mg	30-pk.	26029
RDX	Extraction of explosive compounds (EPA Method 8330) from water samples.	6mL, 500mg	30-pk.	26093

All cartridges are polypropylene and have polyethylene frits.

Cartridges may be processed by positive pressure, sidearm flask, centrifuge, or vacuum manifold.

Analytical Reference Materials

EPA Method 8310 Quality Control Check

(18 components)

acenaphthene	100µg/mL	dibenzo(a,h)anthracene	10
acenaphthylene	100	fluoranthene	10
anthracene	100	fluorene	100
benzo(a)anthracene	10	indeno(1,2,3-cd)pyrene	10
benzo(a)pyrene	10	1-methylnaphthalene	100
benzo(b)fluoranthene	10	2-methylnaphthalene	100
benzo(ghi)perylene	10	naphthalene	100
benzo(k)fluoranthene	5	phenanthrene	100
chrysene	10	pyrene	10

In acetonitrile, 1mL/ampul

cat. # 31843 (ea.)

EPA Method 8310 PAH Mixture (18 components)

500µg/mL each component listed for cat.# 31843 in acetonitrile, 1mL/ampul

cat. # 31841 (ea.)

531.1 Carbamate Pesticide Calibration Mixture

(10 components)

aldicarb	3-hydroxycarbofuran
aldicarb sulfone	methiocarb
aldicarb sulfoxide	methomyl
carbaryl (Sevin®)	oxamyl
carbofuran	propoxur (Baygon®)

100µg/mL each in methanol, 1mL/ampul

cat. # 32273 (ea.)

531.2 Carbamate Pesticide Calibration Mixture

(11 components)

aldicarb	methiocarb
aldicarb sulfone	methomyl
aldicarb sulfoxide	1-naphthol
carbaryl (Sevin®)	oxamyl
carbofuran	propoxur (Baygon®)
3-hydroxycarbofuran	

100µg/mL in acetonitrile, 1mL/ampul

cat. # 32435 (ea.)

8330 Calibration Mix #1 (7 components)

1,3-dinitrobenzene	RDX
2,4-dinitrotoluene	1,3,5-trinitrobenzene
HMX	2,4,6-trinitrotoluene
nitrobenzene	

1,000µg/mL each in acetonitrile, 1mL/ampul

cat. # 31450 (ea.)

8330 Calibration Mix #2 (7 components)

2-amino-4,6-dinitrotoluene	3-nitrotoluene
4-amino-2,6-dinitrotoluene	4-nitrotoluene
2,6-dinitrotoluene	tetryl
2-nitrotoluene	

1,000µg/mL each in acetonitrile, 1mL/ampul

cat. # 31451 (ea.)

Chlorinated Acids by HPLC, Mix B (8 components)

2,4-DB	MCPP (mecoprop)
3,5-dichlorobenzoic acid	4-nitrophenol
dinoseb	pentachlorophenol
MCPA	2,4,5-T

1,000µg/mL each in acetonitrile, 1mL/ampul

cat. # 32430 (ea.)

Chlorinated Acids by HPLC, Mix A (8 components)

acifluorfen (Blazer®)	dicamba
bentazon	dichlorprop
chloramben	picloram
2,4-D	2,4,5-TP (Silvex)

1,000µg/mL each in acetonitrile, 1mL/ampul

cat. # 32431 (ea.)

Dalapon (2,2-dichloropropionic acid)

1,000µg/mL in acetonitrile, 1mL/ampul

cat. # 32432 (ea.)

Chlorinated Acid Herbicide Mix

2,4-dichlorophenoxyacetic acid
2,4,5-TP (Silvex)

1,000µg/mL each in acetonitrile, 1mL/ampul

cat. # 32429 (ea.)

Aldehyde-Ketone-DNPH TO-11A Calibration Mix

(15 components)

acetaldehyde-DNPH	formaldehyde-DNPH
acetone-DNPH	hexaldehyde-DNPH
acrolein-DNPH	isovaleraldehyde-DNPH
benzaldehyde-DNPH	propionaldehyde-DNPH
n-butyraldehyde-DNPH	*m*-tolualdehyde-DNPH
crotonaldehyde-DNPH	*o*-tolualdehyde-DNPH
2,5-dimethylbenz-aldehyde-DNPH	*p*-tolualdehyde-DNPH
	valeraldehyde-DNPH

15µg/mL* each in acetonitrile, 1mL/ampul

cat. # 31808 (ea.)

*Concentration calculated as aldehyde.

Paraquat & Diquat Calibration Mix

diquat dibromide paraquat dichloride

1,000µg/mL each in water, 1mL/ampul

cat. # 32437 (ea.)

Ultra Quat Reagent Solution

Use with Ultra Quat HPLC column. Dilute to 1 liter mobile phase, per instructions.

In water, 20mL/bottle

cat. # 32441 (ea.)

for **more** info

For many additional reference mixes, or for information about custom-prepared mixes, please refer to our catalog or visit our website.