Increase Sample Throughput for Organochlorine Pesticides Analysis with Improved Cleanup and Accelerated Analytical Conditions

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Abstract
Sample throughput limitations for organochlorine pesticides analysis can delay reporting and create bottlenecks in the lab. However, the method described here improves efficiency and productivity by using a two-pronged approach. CarboPrep Plus SPE was used for a more effective sample extract cleanup that reduced inlet contamination and allowed more samples to be run before maintenance was required. In addition, a dual column setup was used with a GC Accelerator oven insert kit in order to speed up overall run times and allow simultaneous confirmation and quantification.

Introduction

In many areas of the world, chlorinated (organochlorine) pesticide use has been banned due to human health and environmental concerns, but these compounds are still widely monitored because of their persistence in the environment. Both soil and wastewater samples are routinely tested to verify the presence or absence of organochlorine pesticides and to assess the risk of exposure from historical contamination.

Organochlorine pesticides analysis can be accomplished by GC using an electron capture detector (ECD) or a mass spectrometer (MS). However, analysis can be challenging because some of the target compounds break down when exposed to active sites in the hot inlet. These active sites are typically caused by nonvolatile sample matrix interferences that are coextracted and then injected along with the target analytes. Once deposited in the inlet and on the head of the analytical column, they foul the system and cause QC failures that lead to down-time for maintenance. SPE cleanup can remove some matrix components from sample extracts, but the Florisil material that is often used is limited in its ability to remove the high molecular weight contaminants that typically cause the problems.

Here, we demonstrate a more effective and efficient approach for organochlorine pesticides analysis. First, Resprep CarboPrep Plus SPE cartridges were used for sample extract cleanup because the graphitized carbon material removes more high molecular weight contaminants than Florisil SPE while still following the same procedure. Second, a GC Accelerator oven insert kit was used along with an Rtx-CLPesticides and Rtx-CLPesticides2 column set for fast, simultaneous confirmation and quantification. Using this approach, labs can increase sample throughput both by removing the nonvolatile matrix compounds that cause QC failures and by speeding up overall analysis times.

Experimental

Sample Extraction

To extract the samples, 10 g of soil was weighted into 50 mL centrifuge tubes containing 5 grams of anhydrous magnesium sulfate. Then, 20 mL of acetone:methylene chloride (50:50) was added, and the samples were centrifuged at 3000 g for five minutes.

Sample Extract Cleanup

Prior to analysis, matrix interferences were removed from the sample extracts using SPE cleanup. For this work, two cleanup options were compared: Resprep Florisil normal phase SPE cartridges (6 mL/1 gm; cat.# 28993) and Resprep CarboPrep Plus graphitized carbon SPE cartridges (3 mL/95 mg; cat.# 25845).

CarboPrep Plus SPE cartridges are designed to use the same solvents and volumes as a standard Florisil cleanup, so the same procedure was employed for both SPE cartridge types (Figure 1). First, one cartridge volume of hexane:acetone (90:10) was added and held for five minutes to condition and equilibrate the beds. Then, using a 1 mL Class A pipette, 1 mL of sample extract was dispensed into each cartridge. The sample extract was loaded until the level matched the upper frit, and the eluent was collected dropwise into a 10 mL volumetric flask. Once the sample was completely loaded, it was immediately followed by the addition of 1 mL of hexane:acetone (90:10), which was also drawn down to the level of the top frit. Then, an additional 8 mL of solvent was added to the cartridges and eluted dropwise into the flask.

Instrumental Analysis

Organochlorine pesticides analysis was performed using a dual column system connected to a single guard column and inlet (Figure 2). The Rtx-CLPesticides and Rtx-CLPesticides2 column set was selected because the stationary phase differences allow confirmation and quantification analyses to be conducted simultaneously using a single injection of sample extract. In this setup, the inlet can be run in either split or splitless mode, depending on reporting limit requirements, but splitless injection was used here.

In addition, a GC Accelerator oven insert kit (cat.# 23849) was installed to speed up analysis. By reducing oven volume, these inserts allow faster ramp rates to be attained, which reduces oven cycle time and allows for increased sample throughput. In order to fit the inserts into the GC oven, analytical columns that were not wound onto cages were used. This customized format can be obtained from Restek simply by ordering the standard column catalog number and specifying "-051" under special instructions.

The detector used for organochlorine pesticides analysis of soil sample extracts processed through both SPE cleanup materials was a micro-ECD.

Figure 1: CarboPrep Plus SPE uses the same cleanup method as a standard Florisil SPE procedure.

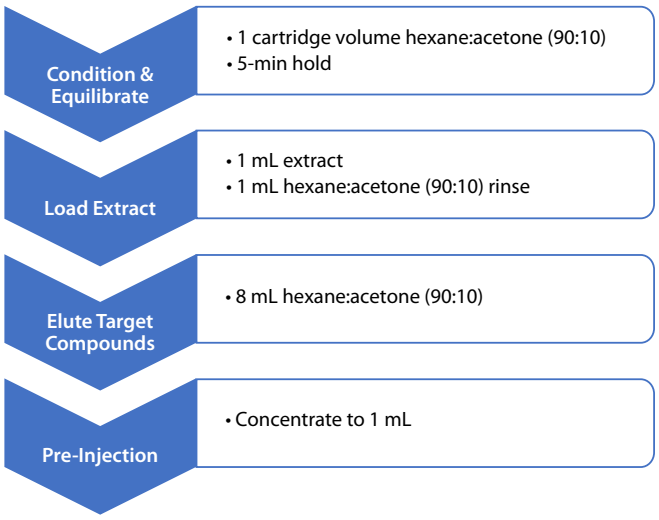
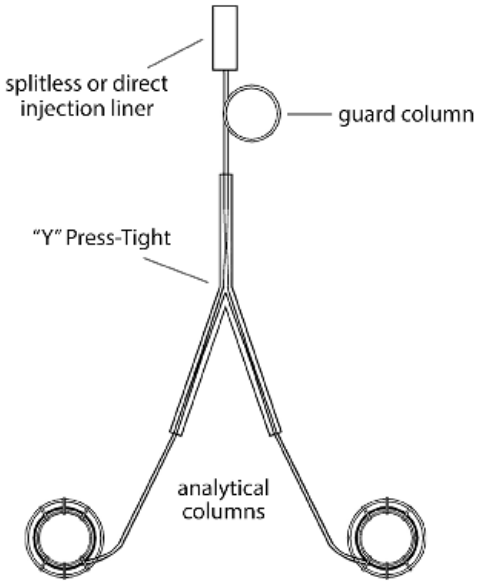


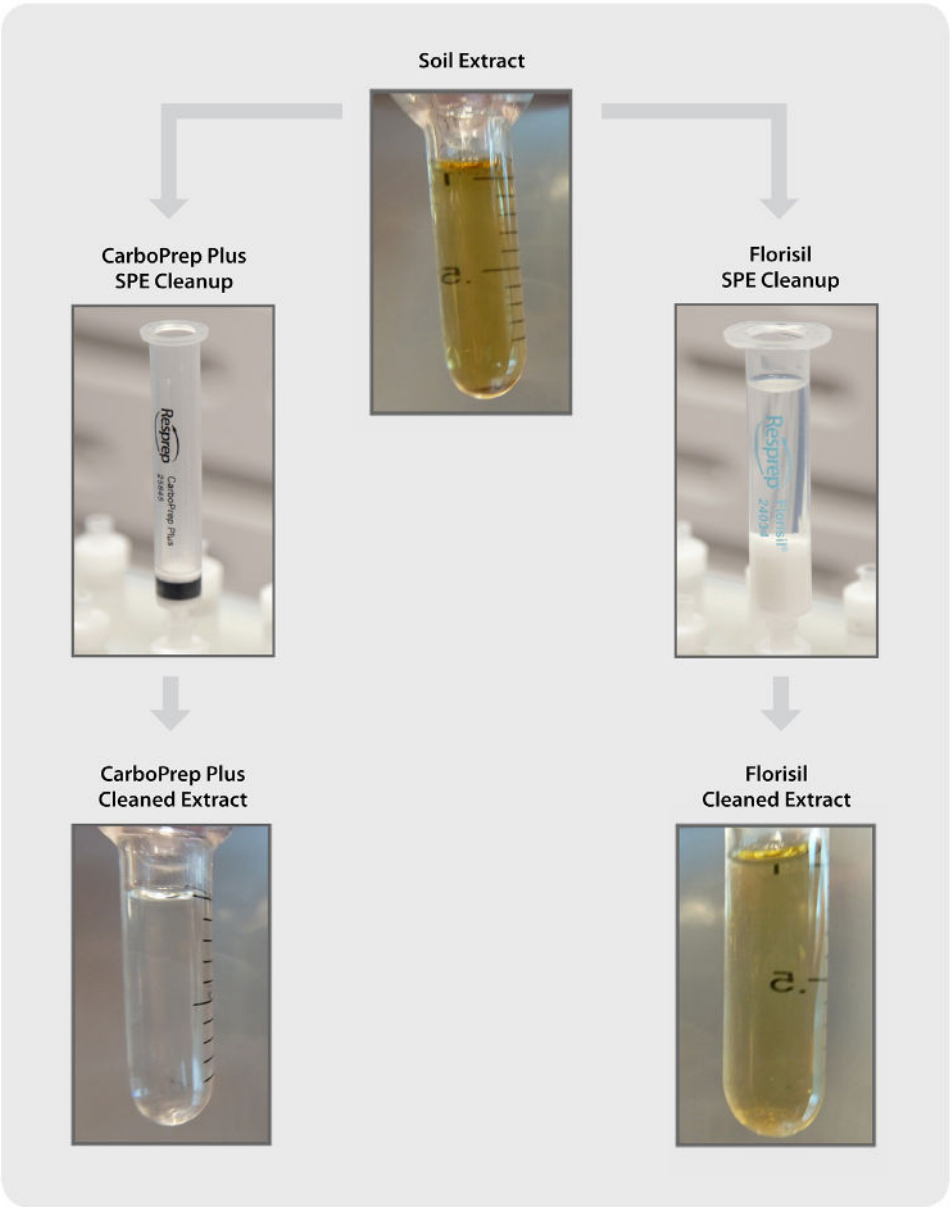
Figure 2: Instrument setup for dual column analysis from a single injection port.



Results and Discussion

The first difference observed in the SPE cleanups is the color of the final extracts. The initial extract is dark because it contains a substantial amount of nonvolatile material from the soil sample. As shown in Figure 3, the Florisil cleanup removes some of these high molecular weight compounds, but overall the final extract is still quite dark, indicating high levels of contaminants remain. In contrast, the extract cleaned with Carboprep Plus SPE is very clear, visually demonstrating its ability to remove much more of the coextracted matrix components.

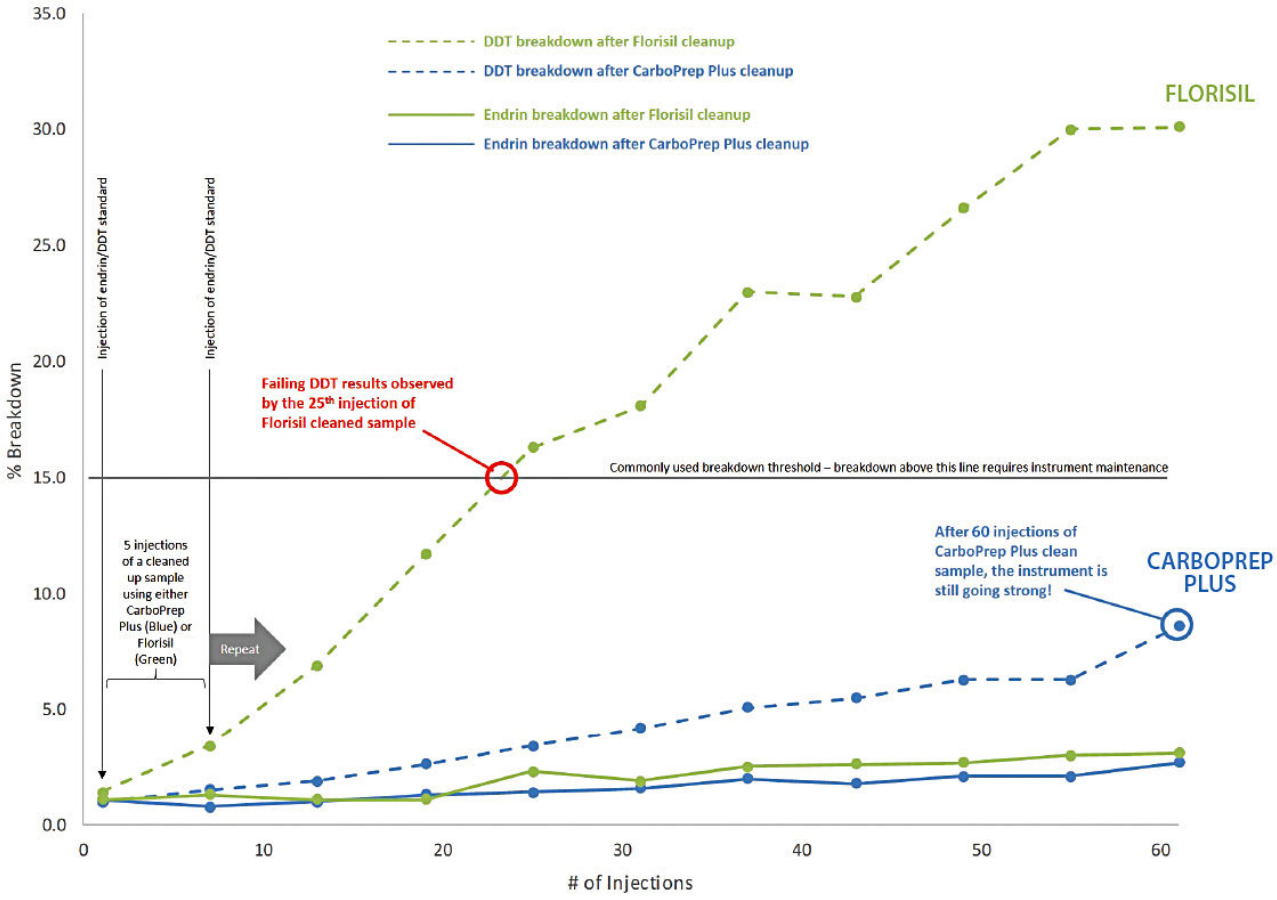
Figure 3: Using the same sample prep process, CarboPrep Plus SPE cleanup removes more coextracted matrix components than Florisil SPE cleanup.



While it is visually clear the CarboPrep Plus SPE cleanup removed more nonvolatile matrix components than the standard Florisil cleanup that is typically used for organochlorine pesticides analysis, the impact on chromatographic performance must also be assessed. To investigate this, we monitored endrin and DDT breakdown as indicators of system suitability using the typical failure threshold of >15%. As shown in Figure 4, the Florisil cleaned extract showed unacceptable 4,4'-DDT degradation (>15%) after just 25 injections, meaning maintenance would be required before analysis could continue. In contrast, the Carboprep Plus cleaned extract was <5% at 25 injections and <10% breakdown after 50 injections. Further, good recoveries were obtained for all target analytes in the extracts cleaned using CarboPrep Plus SPE, indicating that breakdown in the inlet was not occurring (Table I)

The significance of this is that by cleaning extracts more effectively, inlet contamination is substantially reduced so more samples can be run in a calibration sequence before instrument maintenance is required. Fewer failed calibrations, more instrument uptime, and less frequent maintenance help labs increase sample throughput and allow faster turnaround times for data reporting.

Figure 4: CarboPrep Plus cleanup kept the instrument running more than twice as long compared to when analyzing Florisil cleaned samples on the same instrument.



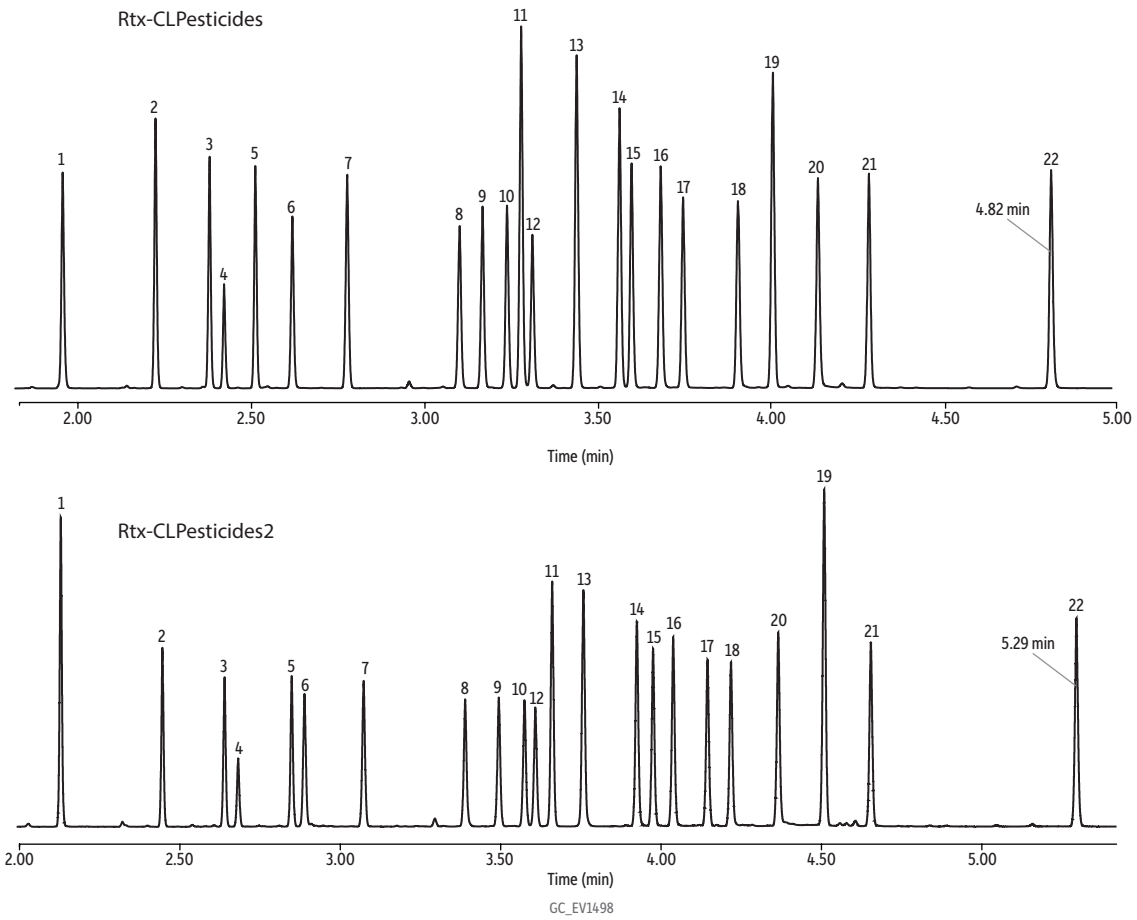
Endrin (50 ppb) and DDT (100 ppb) breakdown measured between injections of an environmental sample extract cleaned up using either Forisil or CarboPrep Plus cartridges.

Table I: Recovery of chlorinated pesticides using CarboPrep Plus SPE.

Compound	Average %Recovery*	%RSD
TCMX	99.8	1.3
Hexachlorobenzene	97.6	2.3
alpha-BHC	99.2	1.3
gamma-BHC	98.1	1.4
beta-BHC	98.7	1.4
delta-BHC	98.7	1.7
Heptachlor	101.2	1.7
Aldrin	97.1	1.7
Heptachlor epoxide	98.6	1.6
gamma-Chlordane	98.2	1.8
alpha-Chlordane	96.9	2.2
4,4'-DDE	96.8	1.6
Endosulfan I	98.0	1.4
Dieldrin	97.8	1.4
Endrin	98.4	1.6
4,4'-DDD	97.4	1.4
Endosulfan II	94.6	5.5
4,4'-DDT	98.2	1.2
Endrin aldehyde	96.1	1.2
Methoxychlor	100.2	2.1
Endosulfan sulfate	97.1	2.2
Endrin ketone	98.1	0.8
DCB	97.8	2.2
* n = the analysis of 6 cartridges each from 3 different lots for a total of 18 data points		

In addition to the productivity gains made by using more effective extract cleanup, speed gains were also made in terms of the overall organochlorine pesticides analysis. The dual column system setup using the Rtx-CLPesticides and Rtx-CLPesticides2 columns provides simultaneous confirmation and quantitation with the elution order switches of 4,4'-DDE with Endosulfan I and of methoxychlor with endosulfan sulfate (Figure 5). Use of a GC Accelerator oven insert kit reduced the total analysis cycle time to around 10 min with the last compound eluting at 4.82 min and 5.29 min on the Rtx-CLPesticides and the Rtx-CLPesticides2 columns, respectively.

Figure 5: Organochlorine pesticides analysis of a prepared standard.



Peaks	Conc. (ng/mL)
1. Tetrachloro-*m*-xylene	25
2. α-BHC	25
3. γ-BHC	25
4. β-BHC	25
5. δ-BHC	25
6. Heptachlor	25
7. Aldrin	25
8. Heptachlor epoxide	25
9. *trans*-Chlordane	25
10. *cis*-Chlordane	25
11. 4,4'-DDE	50
12. Endosulfan I	50
13. Dieldrin	50
14. Endrin	50
15. 4,4'-DDD	50
16. Endosulfan II	50
17. 4,4'-DDT	50
18. Endrin aldehyde	50
19. Methoxychlor	250
20. Endosulfan sulfate	50
21. Endrin ketone	50
22. Decachlorobiphenyl	50

Columns	Rtx-CLPesticides 30 m, 0.32 mm ID, 0.32 µm (cat.# 11141-051) and Rtx-CLPesticides2 30 m, 0.32 mm ID, 0.25 µm (cat.# 11324-051) using Rxi guard column 5 m, 0.32 mm ID (cat.# 10039) with universal angled "Y" Press-Tight connector (cat.# 20403-261)
Sample	Organochlorine pesticide mix AB #2 (cat.# 32292) Pesticide surrogate mix (cat.# 32453)
Diluent:	*n*-Hexane
Injection	
Inj. Vol.:	2 µL splitless (hold 0.3 min)
Liner:	Topaz 4.0 mm ID single taper inlet liner w/wool (cat.# 23303)
Inj. Temp.:	250 °C
Purge Flow:	40 mL/min
Oven	
Oven Temp.:	120 °C (hold 0.3 min) to 200 °C at 60 °C/min to 230 °C at 25 °C/min to 320 °C at 35 °C/min (hold 1.5 min)
Carrier Gas	H_2, constant flow
Linear Velocity:	90 cm/sec
Detector	Micro-ECD @ 330 °C
Make-up Gas	
Flow Rate:	60 mL/min
Make-up Gas Type:	N_2
Data Rate:	50 Hz
Instrument	Agilent 7890A GC
Notes	A GC Accelerator oven insert kit (cat.# 23849) was installed in the instrument to speed up analysis. The analytical columns were custom products that were not on cages. This customized format can be obtained from Restek simply by ordering the standard column catalog number and specifying "-051" under special instructions.

Conclusion

Laboratories are typically under pressure to report data in a timely manner, and unexpected instrument downtime for maintenance significantly reduces productivity. The organochlorine pesticides analysis workflow demonstrated here can help increase sample throughput using two approaches: (1) more effective sample extract cleanup, and (2) simultaneous dual column analysis with oven inserts. Removing more matrix interferences with CarboPrep Plus SPE reduces active sites in the inlet, which allows for longer run sequences and fewer failing continuing calibration standards. In addition, using a dual column analytical approach with the speed gains afforded by GC Accelerator oven inserts helps labs analyze and report results for more samples in less time.

Resprep CarboPrep Plus SPE Cartridges

- Designed specifically for the cleanup of sample extracts for organochlorine pesticides analysis.
- Excellent alternative to Florisil products, especially for the removal of nonvolatile matrix components that contaminate GC inlets and columns.
- Proprietary treatment renders the carbon consistent and clean, ensuring the same selectivity tube to tube, and lot to lot with no interfering background.
- Uses the same hardware, solvents, and solvent volumes as traditional Florisil cleanup, so switching is simple.

25845

Description	Packing	Volume	qty.	cat.#
Resprep CarboPrep Plus SPE Cartridges	CarboPrep Plus	3 mL, 95 mg	30-pk.	25845

Q-sep Multispeed Centrifuge for QuEChERS

- Program 10 custom cycles for time, braking, and speed or g-force (up to 4500 rpm or 3450 xg).
- QuEChERS-specific presets for AOAC and EN methods make consistent operation quick and simple.
- Convenient lid lighting indicates at a glance if unit is ready, running, or done.
- Control panel can be temporarily locked on one cycle for error-free reproducibility.
- Cool-Flow design prevents samples from overheating by maintaining unit at room temperature.
- Tube holders are carbon fiber for high strength, durability, and years of trouble-free use.
- Clear lid permits safe observation of samples and optical calibration of speed.

28295

Intended Use

General-purpose laboratory centrifuge intended for safe and rapid density-based separation of fluids, including physiologic fluids, in approved specimen receptacles for qualitative or quantitative test procedures. As a general-purpose laboratory centrifuge, it is designed to also run other approved containers filled with chemicals (nonflammable, nonexplosive, nonvolatile, and non-highly reactive only), environmental samples, and other nonhuman body samples. This device is intended to be operated by properly trained personnel who have carefully read the operating manual and are familiar with the function of the device.

Description	Includes	Certification/Compliance	qty.	cat.#
Q-sep Multispeed Centrifuge for QuEChERS	15 mL four-place tube holder (6); 50 mL single-place tube holder (6); 50 mL conical tube insert (6); 2 mL tube adaptors (24); U.S. power cord (1); global/universal power cord (1)	UL61010-1/CSA C22.2 No. 61010-1 and IEC61010-2-020; FDA listed; MET U.S. E112532; CE; RoHS	ea.	28295

Specifications

Tube Capacity	6 x 50 mL tubes 18 x 15 mL tubes 24 x 2 mL tubes
Dimensions (H x W x D)	9 in x 14.5 in x 17 in (23 cm x 37 cm x 43 cm)
Weight	39 lb (17 kg)
Sound Level	64 dB A
Environmental Range	16–32 °C
Voltage	95–253 VAC
Frequency	50/60 Hz
Power Requirement	220 Watts
Centrifuge Motor	1/2 H.P. Brushless DC
Max g-Force	3450 xg
Max Speed	4500 RPM
Cycle Time	30 sec to 99 min, 59 sec (±2%)

Empty Centrifuge Tubes, Polypropylene

Description	qty.	cat.#
Empty 50 mL Centrifuge Tube, Polypropylene w/Blue Cap	50-pk.	25846

25846

Rtx-CLPesticides and Rtx-CLPesticides2 Columns (fused silica)

proprietary Crossbond phase

ID	df	Length	Temp. Limits	qty.	Similar to Part #	cat.#
Rtx-CLPesticides Column						
0.32 mm	0.32 µm	30 m	-60 to 320/340 °C	ea.		11141
Rtx-CLPesticides2 Column						
0.32 mm	0.25 µm	30 m	-60 to 320/340 °C	ea.	Agilent 123-1236; Scion/Bruker/Varian CP7936	11324

ordering notes

The analysis in this application note featured the Rtx-CLPesticides and Rtx-CLPesticdes2 columns that were not wound onto cages. This customized format can be obtained from Restek simply by ordering the standard column catalog number and specifying "-051" under special instructions.

Rxi Guard/Retention Gap Columns (fused silica)

- Extend column lifetime.
- Excellent inertness—obtain lower detection limits for active compounds.
- Sharper chromatographic peaks by utilizing retention gap technology.
- Maximum temperature: 360 °C.

ID	Length	OD	qty.	Similar to Part #	cat.#
0.32 mm	5 m	0.45 ± 0.04 mm	ea.	Agilent 160-2325-5; Phenomenex 7AM-G000-00-GHO	10039

20403

Universal Angled "Y" Press-Tight Connectors

- Split sample flow—perform dual-column confirmation analysis with a single injection.
- Split column flow to two detectors.
- Inlet and outlet ends match column curvature to alleviate connection strain.

Description	Deactivation	qty.	cat.#
Universal Angled "Y" Press-Tight Connector	Deactivated	ea.	20403-261
	Deactivated	3-pk.	20404-261

23303

Topaz 4.0 mm ID Single Taper Inlet Liner w/ Wool

for Agilent GCs equipped with split/splitless inlets

ID x OD x Length	Packing	qty	Similar to Part #	cat.#
Single Taper, Premium Deactivation, Borosilicate Glass				
4.0 mm x 6.5 mm x 78.5 mm	Quartz Wool	5-pk.	Agilent 5062-3587 (ea.); 5183-4693 (5-pk.); 5183-4694 (25-pk.); 5190-2293 (ea.); 5190-3163 (5-pk.); 5190-3167 (25-pk.); 5190-3171 (100-pk.)	23303

* 100% SATISFACTION GUARANTEE: If your Topaz inlet liner does not perform to your expectations for any reason, simply contact Restek Technical Service or your local Restek representative and provide a sample chromatogram showing the problem. If our GC experts are not able to quickly and completely resolve the issue to your satisfaction, you will be given an account credit or replacement product (same cat.#) along with instructions for returning any unopened product. (Do not return product prior to receiving authorization.) For additional details about Restek's return policy, visit www.restek.com/warranty

Organochlorine Pesticide Mix AB #2

(20 components)

Aldrin (309-00-2), 8 µg/mL
α-BHC (319-84-6), 8 µg/mL
α-BHC (319-85-7), 8 µg/mL
α-BHC (319-86-8), 8 µg/mL
α-BHC (Lindane) (58-89-9), 8 µg/mL
cis-Chlordane (5103-71-9), 8 µg/mL
trans-Chlordane (5103-74-2), 8 µg/mL
4,4'-DDD (72-54-8), 16 µg/mL
4,4'-DDE (72-55-9), 16 µg/mL
4,4'-DDT (50-29-3), 16 µg/mL
Dieldrin (60-57-1), 16 µg/mL
Endosulfan I (959-98-8), 8 µg/mL
Endosulfan II (33213-65-9), 16 µg/mL
Endosulfan sulfate (1031-07-8), 16 µg/mL
Endrin (72-20-8), 16 µg/mL
Endrin aldehyde (7421-93-4), 16 µg/mL
Endrin ketone (53494-70-5), 16 µg/mL
Heptachlor (76-44-8), 8 µg/mL
Heptachlor epoxide (isomer B) (1024-57-3), 8 µg/mL
Methoxychlor (72-43-5), 80 µg/mL

Conc. in Solvent	CRM?	Min Shelf Life on Ship Date	Max Shelf Life on Ship Date	Shipping Conditions	Storage Temp.	qty.	cat.#
Organochlorine Pesticide Mix AB #2							
In hexane:toluene (1:1), 1 mL/ampul	Yes	6 months	48 months	Ambient	10 °C or colder	ea.	32292

32292

Pesticide Surrogate Mix

(2 components)

Decachlorobiphenyl (2051-24-3), 200 µg/mL
2,4,5,6-Tetrachloro-*m*-xylene (877-09-8), 100 µg/mL

Conc. in Solvent	CRM?	Min Shelf Life on Ship Date	Max Shelf Life on Ship Date	Shipping Conditions	Storage Temp.	qty.	cat.#
Pesticide Surrogate Mix							
In acetone, 1 mL/ampul	Yes	6 months	75 months	Ambient	0 °C or colder	ea.	32453

GC Accelerator Oven Insert Kit

for Agilent 5890, 6890, 7890, and 8890 instruments

- Get the same GC separation in less time—use a GC Accelerator kit and the *EZ*GC method translator to accurately convert methods to a scaled-down column format.
- Scaled-down methods let you speed up analysis time and increase sample throughput without capital investment.
- GC Accelerator kit installs easily without damaging the GC column or interfering with the MS interface.

23849

Description	Instrument	qty.	cat.#
GC Accelerator Oven Insert Kit	for Agilent 5890, 6890, 7890, and 8890 instruments	kit	23849

RESTEK
Pure Chromatography

Questions? Contact us or your local Restek representative (www.restek.com/contact-us).

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