

dSPE Sample Clean-up

**cat.#s 26215, 26216, 26217, 26218, 26124, 26125, 26123,
26219, 26220, 26221, 26222, 26223, 26224, 26225, 26226, 26126**

Dispersive solid phase extraction (dSPE) is one technique used for sample cleanup. Sample cleanup often is preceded by sample extraction; extraction methods vary from traditional liquid-liquid extraction to cartridge solid phase extraction. This Restek dSPE product can be used as an independent clean-up tool or as part of a QuEChERS method.

Quick, Easy, Cheap, Effective, Rugged, and Safe, the QuEChERS (“catchers”) method is based on work done and published by the US Department of Agriculture Eastern Regional Research Center in Wyndmoor, PA.¹ Researchers developed a new extraction method for pesticides in fruits and vegetables, coupled with a clean-up method that removes sugars, lipids, organic acids, sterols, proteins, pigments and excess water. This technique offers a user-friendly alternative to traditional liquid-liquid and solid phase extractions.

The procedure involves two simple steps. First, the homogenized samples are extracted and partitioned using an organic solvent and salt solution. Then, the supernatant is further extracted and cleaned using a **dispersive solid phase extraction (dSPE) technique**.

Restek products make this approach even simpler. We offer QuEChERS extraction and dSPE products in a variety of standard sizes and formats. The dSPE centrifuge tube format (available in 2mL and 15mL sizes) contains magnesium sulfate (to partition water from organic solvent) and primary secondary amine (PSA) adsorbent (to remove sugars and fatty acids). These tubes are available with or without graphitized carbon (to remove pigments and sterols) and/or C18 packing (to remove nonpolar interferences such as lipids).

Several detailed QuEChERS methods have been published and are listed below. Restek dSPE tubes, listed in Table I (back page), are formulated according to these methods.

- **Mini-Multiresidue Method**

QuEChERS-A Mini-Multiresidue Method for the Analysis of Pesticide Residues in Low-Fat Products²

- **AOAC Official 2007.01 Method**

Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate³

- **European prEN_15662 Method, Version 2007-10-24**

Foods of Plant Origin—Determination of Pesticide Residues Using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Clean-up by Dispersive SPE—QuEChERS-method⁴

QuEChERS-based methods have several basic steps in common, which are described below. Specific procedures for sample extraction and dSPE sample clean-up according to *QuEChERS-A Mini-Multiresidue Method for the Analysis of Pesticide Residues in Low-Fat Products*² are given in the following sections.



General Procedures (common for all 3 QuEChERS-based methods listed above)

Step 1: Sample preparation and extraction

Commodities are uniformly ground. Internal standards are also added at this point. Various salts, acids and buffers may then be added to enhance extraction efficiency and protect sensitive analytes.

Step 2: Sample extract cleanup

A subsample of the modified solvent extract from Step 1 is cleaned up using dSPE. Small polypropylene centrifuge tubes are pre-filled with precise weights and proportions of bulk drying salts and SPE adsorbent packings to remove excess water and unwanted contaminants from the sample extracts. After a brief agitation and centrifugation, the cleaned extracts are then prepared for analysis.

Step 3: Sample analysis

Samples may be pH adjusted, solvent-exchanged, or treated with additional agents, to protect sensitive analytes or improve analysis by either GC/MS or LC/MS.

dSPE Sample Clean-up

These instructions focus on dSPE sample clean-up procedures. *Sample extraction precedes dSPE clean-up and generalized extraction instructions are provided in the Sample Extraction, Sample Drying and Buffering, and Phase Separation sections.*

The sample can be analyzed directly from the raw extract, especially if pesticides with acidic groups (e.g. phenoxyacid herbicides) are of interest. Alternatively, sample clean-up methods can be applied. Specifically, dispersive solid phase extraction is discussed here. Restek dSPE tubes are formulated in accordance with published methods and are listed in Table I. Select tubes based on the method and sample type; general guidelines for different sample types include:

For samples with co-extracted fats or waxes:

Before or after clean-up, samples are put in freezer (>1hr. to overnight). Cold samples are then re-centrifuged and fats or waxes are removed. If fat remains, clean up PSA, MgSO₄, C18 tubes is recommended. If no fat remains, PSA and MgSO₄ tubes are recommended.

For samples with remaining fats:

Clean up with PSA, MgSO₄, and C18 tubes is recommended (see above).

For samples with colored extracts or high carotinoid or chlorophyll levels:

Clean up with PSA, MgSO₄, and graphitized carbon tubes is recommended.

For samples with colored extracts containing greater than 1% fat:

Clean up with PSA, MgSO₄, C18, and graphitized carbon tubes is recommended.

For all other samples:

Clean up with PSA and MgSO₄ tubes is recommended.



Once tubes are selected, dSPE sample clean-up can be accomplished according to the following procedure, which are based on *QuEChERS-A Mini-Multiresidue Method for the Analysis of Pesticide Residues in Low-Fat Products*². For complete information, refer to the original source method at www.quechers.de or www.quechers.com:

1. Using the centrifuged extracts resulting from the **phase separation stage of sample extract preparation**, transfer the supernatant to the dSPE tube as shown in Figure 1. Use Table I to determine the volume of sample that should be transferred.
2. Shake vigorously for 30 seconds or 2 minutes (Figure 2). Use Table I to determine the suggested shake time.
3. Centrifuge for 5 minutes at 3,000U/min. to separate the solid material (Figure 3).
4. Immediately adjust the supernatant pH using a 5% formic acid in acetonitrile solution. Use 10µL **per mL** of supernatant. For sulfonyl urea herbicides, carbosulfan, and benfuracarb, analyze the supernatant without any pH adjustment.
5. Transfer sample to an autosampler vial and test using GC or LC methods (Figure 4).

Sample Extraction

1. Homogenize the frozen commodity to generate a uniform sample representative of the product (Figure 5). For products with more than 5% fat (w/w), see the specialized sample preparation procedure in the source method, *QuEChERS-A Mini Multiresidue Method for the Analysis of Pesticide Residues in Low-Fat Products*.²
2. Weigh 10g of homogenized product into a clean 50mL tube (cat.#26227) as shown in Figure 6.
3. Add 10mL acetonitrile and an appropriate amount of an internal standard solution. See suggestions of internal standards and volume in the source method.²
4. Shake vigorously for 1 minute by hand (Figure 7).



Sample Drying and Buffering

1. Add the contents (listed below) of a Resprep™ Q110 tube (cat.# 26213) to each extracted sample (Figure 8).

4.0g ± 0.2g	magnesium sulfate, anhydrous
1.0g ± 0.05	sodium chloride
1.0g ± 0.05g	trisodium citrate dihydrate
0.5g ± 0.03g	disodium hydrogencitrate sesquihydrate

Note:

If pH <3, sample should be adjusted with addition of 600µL of 5N NaOH (lemons, limes, currants).
If pH >3 and <5, sample should be adjusted with 200µL of 5N NaOH (raspberries).

2. Shake immediately and vigorously 1 minute (Figure 7).



Phase Separation

Centrifuge for 5 minutes at 3,000 U/min. to separate the solid material (Figure 9). Proceed with dSPE sample clean-up or analyze extract directly without clean-up.

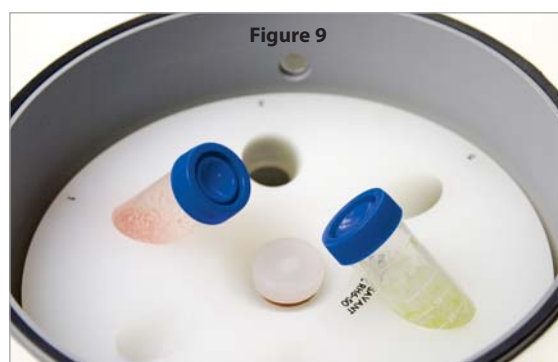


Table I Restek dSPE tubes (organized by published method).

Cat.#	Name	Centrifuge Tube		Method	Sample Volume (mL)	Shake Time (min.)	Centrifuge Speed	Centrifuge Time (min.)
		Size (mL)	Contains					
26216	Resprep Q211	2	150mg MgSO ₄ , 25mg PSA, 25mg C18	Mini-Multiresidue	1	0.5	3,000 U/min.	5
26215	Resprep Q210	2	150mg MgSO ₄ , 25mg PSA	Mini-Multiresidue, European prEN-15662	1	0.5	3,000 U/min.	5
26217	Resprep Q212	2	150mg MgSO ₄ , 25mg PSA, 2.5mg GCB	Mini-Multiresidue, European prEN-15662	1	2	3,000 U/min.	5
26218	Resprep Q213	2	150mg MgSO ₄ , 25mg PSA, 7.5mg GCB	Mini-Multiresidue, European prEN-15662	1	2	3,000 U/min.	5
26223	Resprep Q370	15	900mg MgSO ₄ , 150mg PSA	European prEN-15662	6	0.5	3,000 U/min.	5
26224	Resprep Q371	15	900mg MgSO ₄ , 150mg PSA, 15mg GCB	European prEN-15662	6	2	3,000 U/min.	5
26225	Resprep Q372	15	900mg MgSO ₄ , 150mg PSA, 45mg GCB	European prEN-15662	6	2	3,000 U/min.	5
26124	Resprep Q250	2	150mg MgSO ₄ , 50mg PSA	AOAC 2007.01	1	0.5	>1,500 rcf	1
26125	Resprep Q251	2	150mg MgSO ₄ , 50mg PSA, 50mg C18	AOAC 2007.01	1	0.5	>1,500 rcf	1
26219	Resprep Q252	2	150mg MgSO ₄ , 50mg PSA, 50mg C18, 50mg GCB	AOAC 2007.01	1	0.5	>1,500 rcf	1
26220	Resprep Q350	15	1,200mg MgSO ₄ , 400mg PSA	AOAC 2007.01	8	0.5	>1,500 rcf	1
26221	Resprep Q351	15	1,200mg MgSO ₄ , 400mg PSA, 400mg C18	AOAC 2007.01	8	0.5	>1,500 rcf	1
26222	Resprep Q352	15	1,200mg MgSO ₄ , 400mg PSA, 400mg C18, 400mg GCB	AOAC 2007.01	8	0.5	>1,500 rcf	1
26123	Resprep Q253	2	150mg MgSO ₄ , 50mg PSA, 50mg GCB	AOAC 2007.01	1	2	>1,500 rcf	1
26226	Resprep Q373	15	900mg MgSO ₄ , 150mg PSA, 150mg C18	similar to European prEN-15662	6	0.5	3,000 U/min.	5
26126	Resprep Q374	15	900mg MgSO ₄ , 300mg PSA, 150mg GCB	NA	6	2	3,000 U/min.	5

PSA = primary and secondary exchange material

GCB = graphitized carbon black

Notes:

U/min.= Undrehungen pro minute and is the German unit of revolutions per minute (RPM)

rcf = relative centrifugal force and can be converted to RPM using $rcf = 1.12r \left(\frac{RPM}{1000} \right)^2$
 r = the radius of the centrifuge rotation.

References

1. M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC International 86, p. 412-431 (2003).
2. QuEChERS-A Mini-Multiresidue Method for the Analysis of Pesticide Residues in Low-Fat Products. <http://www.quechers.com> (accessed July 15, 2008).
3. AOAC Official Method 2007.01, Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate.
4. prEN 15662 Version 2007-10-24, Foods of Plant Origin—Determination of Pesticide Residues Using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Clean-up by Dispersive SPE (QuEChERS-method).

