

**This Data Sheet Contains Important Information About The Product.**

**Product Description**

When silver ions are loaded and immobilized on to an SCX phase as counter-ions, they have the ability to form polar complexes with unsaturated fatty acid double bonds under normal-phase conditions. Discovery® Ag-Ion SPE was developed for the fractionation cis/trans isomers, and can also resolve FAMES by degree of unsaturation in which retention strength increases with increasing number of double bonds. As a result, Discovery Ag-Ion SPE allows users to fractionate FAME samples prior to GC analysis thereby simplifying analytical chromatography and improving method accuracy.

Discovery Ag-Ion SPE phases are loaded with silver using a proprietary procedure to offer optimal resolution, performance, and capacity. Each lot is tested and quality controlled for cis/trans FAME resolution.

**Recommend Protocol**

**Sample Extraction:**

Food samples can be initially extracted directly with petroleum ether. Fatty acids are then methylated to FAMES using BF<sub>3</sub> in methanol. FAMES are further extracted into hexane prior to SPE fractionation and capillary GC analysis. Detailed procedures are available from Supelco Technical Service (800-359-3041 or [techservice@sial.com](mailto:techservice@sial.com)).

Alternatively, hydrolytic extraction and methylation procedures described in AOAC 996.06 (1) and AOCS Official Method Ce 2-66 can be used prior to SPE fractionation (2). FAMES should be dissolved in hexane prior to SPE fractionation.

**Discovery Ag-Ion SPE:**

**SPE:** Discovery Ag-Ion SPE Tube, 750 mg/6 mL (54225-U); OR  
Discovery Ag-Ion Rezorian™ SPE Cartridges\* 750 mg/1 mL (54226-U)

**Note:** For all SPE steps, flow rate should be controlled at 5 mL/min., or 2-3 drops per second. The sample/mobile phase should be pulled completely through the cartridge. However, excessive drying of the sorbent should be avoided.

SPE Step:	Description	Comments
1. Condition	Condition SPE with 4 mL acetone.	Moisture adsorbed on the SPE phase may affect SPE performance. Acetone conditioning removes residual moisture from the SPE phase.
2. Equilibrate	Equilibrate cartridge with 4 mL hexane.	
3. Sample Load	Load ≤ 1 mg FAMES in hexane derived from sample extraction at a flow rate of 2-3 drops/second.	Discovery Ag-Ion 750 mg cartridges have a maximum capacity of 1 mg FAMES. Exceeding the capacity will reduce resolution efficiency of the cartridge.
4. Fraction 1	Elute fraction 1 with 6 mL hexane:acetone 96:4	Fraction 1 will target: <ul style="list-style-type: none"> <li>• Saturated fatty acids</li> <li>• Trans monoenes</li> <li>• Cis/cis and trans/trans conjugated linoleic acids (CLAs)</li> </ul>
5. Fraction 2	Elute fraction 2 with 4 mL hexane:acetone 90:10	Fraction 2 will target: <ul style="list-style-type: none"> <li>• Cis monoenes</li> <li>• Trans/trans dienes</li> <li>• Cis/trans and trans/cis CLAs</li> </ul>
6. Fraction 3	Elute fraction 3 with 4 mL acetone	Fraction 3 will target: <ul style="list-style-type: none"> <li>• Cis/cis dienes</li> <li>• Other dienes</li> <li>• Most trienes</li> </ul>
7. Evaporation / Reconstitution	Evaporate all fractions at 40 °C under N <sub>2</sub> sparge. Reconstitute in 0.2 mL-1.0 mL hexane prior to GC analysis	

\*Rezorian cartridges are small polypropylene barrels that are capped at both ends with Luer-Lock® fittings. The hardware is designed for low positive pressure applications. The cartridges can also be adapted for use with vacuum manifolds when used in conjunction with the proper Luer-Lock connectors. Please contact Supelco Technical Service for more information (800-359-3041 or [techservice@sial.com](mailto:techservice@sial.com)).

**Note:** Discovery Ag-Ion SPE should be stored in a desiccated environment upon removal from the metallic mylar packaging.

## Troubleshooting

Observation	Possible Cause	Possible Solution
Incomplete fractionation of 18:1 cis and 18:1 trans isomers. Greater than 5% of total cis isomers co-eluted with trans isomers in fraction 1.	High moisture content of acetone used during conditioning (step 1)	Condition with acetone that has less than 0.5% residual moisture contamination.
	The flow rate during sample load was too high	Decrease the flow rate to 5 mL/min. or 1-2 drops/sec.
	Elution volume is too high.	Decrease elution volume for fraction 1.
18:1 trans isomers were over retained and greater than 5% of 18:1 trans isomers co-eluted with cis-isomers and dienes in fractions 2 and 3	High moisture content of acetone used during conditioning (step 1)	Condition with acetone that has less than 0.5% residual moisture contamination.
	The flow rate during sample load was too high	Decrease the flow rate to 5 mL/min. or 1-2 drops/sec.
	Insufficient elution volume	Increase elution volume for fraction 1
Fractionation of structural isomers was incomplete and/or poor resolution observed b/w monoenes, and dienes.	Sample has too many impurities	Further sample cleanup using additional extraction steps may be necessary.
	High moisture content of acetone used during conditioning (step 1)	Condition with acetone that has less than 0.5% residual moisture contamination.
	The flow rate during sample load was too high	Decrease the flow rate to 5 mL/min. or 1-2 drops/sec.
	FAMEs content of the sample was too high and the SPE cartridge was over loaded.	Estimate the amount of FAMEs and load a maximum of 1 mg FAMEs on to the sorbent.

### References:

1. Official Methods of Analysis of AOAC International, AOAC Official Method 996.06 "Fat (Total, Saturated and Unsaturated) in Foods", 17<sup>th</sup> edition, Revision 1, 2002
2. AOCS Official Method Ce 2-66, Preparation of Methyl Esters of Long-Chain Fatty Acids

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