PRODUCT MANUAL

for

ASE® Prep CR H+ form

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Product Manual

For the

ASE® Prep CR H+ form

CATION EXCHANGE RESIN
400g (P/N 071397)

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SECTION 1 – INTRODUCTION

The ASE® Prep CR H+ form (P/N 071397) is a cation exchange resin consumable in the hydrogen form that is used with the new ASE 350/ 150 systems for determination of total lipids in foods after base hydrolysis. The CR H+ form resin in conjunction with ASE Prep DE (diatomaceous earth) P/N 062819 allows neutralization of base. This manual describes how to use the ASE Prep CR H+ form resin for base hydrolysis work.

Many samples require the use of acid or base solvents to adequately solubilize analytes. There are also samples that require pretreatment with concentrated acids or bases prior to pursuing extraction so that the analytes of interest are available for extraction by organic or aqueous solvent systems. For example, pursuing analysis of lipids in some food samples is difficult since the lipids are complexed with carbohydrate or proteins. Disintegration of the sample with a base and heat, hydrolyzes the proteins and starch, disrupts the bond and liberates the fat to allow for easy extraction. The purpose of the base hydrolysis is to release bound lipids.

Association of Official Analytical Chemists International (AOAC) method 996.06 describes base hydrolysis step for food samples followed by a liquid-liquid extraction method called Mojonnier extraction method. The lipids are extracted away from the base and other matrix components using specific ether based solvent and then the solvent is evaporated leaving the lipids available for gravimetric analysis or further analysis by GC/GC-MS after converting the lipids to the fatty acid methyl ester form.

The Mojonnier method is a liquid extraction method and is time consuming and labor intensive and is subject to operator to operator variance. Additionally the Mojonnier method exposes the practitioner to concentrated acids and solvents by requiring the need to shake the concentrated base solvent mixture.

The Accelerated Solvent Extraction on the other hand is an automated means of pursuing extraction. By neutralizing the concentrated base the CR H+ form resin eliminates exposure of the practitioner to the base concentrates and inline extraction of the lipids became feasible with the ASE instrument.

Table 1
ASE Prep CR H+ form Resin Specifications

Resin	ASE Prep CR H+ form
Particle Diameter (mm)	1.2-0.30
Polymer	Styrene divinylbenzene copolymer
Functional Group	Sulfonic acid
Ionic Form	Hydrogen
Substrate X-Linking %	8
Substrate Type	Macroporous
Exchange capacity (meq/g)	1.8

Assistance is available for any problem during the shipment or operation of Dionex instrumentation, columns, and consumables through the Dionex North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or through any of the Dionex Offices listed in "Dionex Worldwide Offices" on the Dionex Reference Library CD-ROM.

SECTION 2 – LIPID ANALYSIS PROCEDURE

This section describes the steps needed for lipid analysis such as base hydrolysis procedure and ASE extraction procedure.

2.1. Chemicals/Materials Required

- Ammonium hydroxide (NH_4OH ACS grade, 58% w/w, 28-30% NH_3 content, CAS 1336-21-6).
- Ethanol (95% ACS grade).
- Hexane (ACS grade or higher).
- Petroleum ether and diethyl ether (ACS grade).
- ASE® Prep DE (P/N 062819).
- ASE® Prep CR H+ form (P/N 071397).

2.2. Typical food samples for base hydrolysis:

Typical samples analyzed by the base hydrolysis method are diary products such as milk, cream, half & half milk, heavy whipping cream, cream cheese, sour cream, cottage cheese, condensed milk, yogurt.

2.3. Base hydrolysis of food samples-procedure for gravimetric determination:

Weigh out the food samples in a 40 mL (Dionex Corporation, P/N 048783) clear glass bottle/vial. Adjust the amount of sample so that approximately 100-200 mg of lipid is released during hydrolysis (AOAC 996.06). Use an analytical balance and record the weight up to 4 significant figures. Add 2 mL of ethanol into the bottle and mix the contents. Add 4 mL of Water/DI into the bottle and mix the contents. Finally add 2 mL of 58% NH_4OH (= 28-30% NH_3 content, CAS 1336-21-6), tighten/fix the cap and mix the contents thoroughly. The vortex can be used for mixing but with moderate agitation.

WARNING ***It is critical that the entire sample content be submersed in the liquid layer. Do not leave any food particles above the liquid layer inside the vial. Follow all safety procedures while handling the base samples.***

Hydrolyze the sample for 20 min at 70-80 °C (as described in AOAC method 996.06) with constant shaking in a water bath or any other suitable heater placed inside a fume hood

WARNING ***The temperature needs to be controlled at the specified range as stated in AOAC method 996.06. Lower temperature may yield lower lipid/fat content. It is recommended to pursue hydrolysis for a maximum of 20 minutes. It should be noted that pursuing hydrolysis at higher temperature and longer time may disintegrate and destroy the sample leading to poor recovery.***

Take out the sample bottle out of water bath or heater and let the vial cool down for 5 to 10 min. Wipe or clean the out side of the bottle with a paper towel.

Hydrolysis is complete at this point. For ASE extraction we recommend adding 6 mL of ethanol to the sample vial and proceed to the sample preparation procedure prior to ASE extraction.

WARNING ***Add ethanol when vial is cold!***

2.4. Sample preparation procedure for ASE: Standard Resin Method

The extraction of food sample is done in a 100 mL Zirconium extraction cell (P/N 068103). The ASE Prep CR H+ form resin (P/N 071397) can be weighed out in a weighing pan on a balance or resin can be added using the supplied tablespoon (1tbsp, Dionex P/N 068505). The hydrolyzed food sample is mixed with resin and DE and loaded into a 100 mL cell with plugs of resin on the bottom and top. The detail steps are described below.

Figure 1
Table spoon for volumetric measurements
(1 tbsp, P/N 068505).

a. Take a 100 mL Zirconium cell. Fix the bottom cell end cap and insert a cellulose filter (P/N 056780) from the top and push it with filter insertion tool (P/N 056929).

b. Use an aluminum funnel (P/N 056699) on the top of the cell to aid loading. Add a plug (about 5-6 g by wt. or ½ tbsp) of ASE Prep CR H+ form resin to the bottom of the cell. This layer ensures a reservoir of capacity to capture any break through of base through the cell during extraction.

c. Take a pre-cleaned mortar. Add 15-16g of ASE Prep DE (P/N 062819) into the mortar.

WARNING ***Do not take more than 16g DE. If the total mass (DE+resin) is greater than the space inside a 100 mL cell then all the sample can not be loaded into the 100 mL cell.***

d. Grind the DE using a pre-cleaned pestle to break the big DE particles and make some fines.

NOTE ***It is important to grind the DE because the big DE particles can occupy significant volume in the cell thus limiting the amount of sample that can be loaded in the cell.***

e. Add about 15g (or 1 and ½ tbsp) of ASE Prep CR H+ form resin to the mortar and lightly mix the contents with pestle but do not grind the resin into smaller particles.

f. Add the base hydrolyzed sample over this resin and DE mixture evenly in mortar. The hydrolysis sample bottle can be rinsed with 2 mL portion of ethanol in order to rinse out the residual sample. Transfer the contents into the mortar.

g. Mix the contents of the mortar with a lab spatula or spoon lightly and follow this by mixing with the pestle. Break the clumps by using the pestle and mix well but do not grind the ASE Prep CR resin.

h. Load this mixture into the 100 mL cell from step b containing the cellulose filter and the resin layer. Constantly tap the bottom end of the cell on the lab counter while loading the sample mixture to ensure adequate compaction for loading the whole mass from step g.

i. Add another plug (about 5-6 g by wt. or ½ tbsp) of resin to top off the cell and fix the top end cap. The extraction cell is now ready for extraction in the ASE instrument.

2.5. Extraction in ASE:

Load the cell into the ASE instrument (Dionex ASE 350 or 150) upper carousel for extraction at 110 °C using hexane as the extraction solvent. Load the pre-weighed 250 mL collection bottle (P/N 056284) in the lower carousel for collection of the extract. Record the weight of the bottle/bottles. Use the method menu and create a method using the conditions (A) listed below for the extraction (standard mode) and run.

Users, who have ASE350, additional/alternative extractions for solvent saver modes can be pursued for similar lipid recovery as standard mode but with less solvent usage. Use the method menu and create a method using the conditions listed below for the extraction and run.

(A) Standard mode
Solvent Saver: OFF
Pressure: 1500 psi
Temperature: 110 ° C
Heating time: 6 min
Static time: 15 min
Cycles: 1
Flush: 30 % (Recommended)
Purge: 120 sec
Total Time: 26-27 min
Solvent: Hexane

(B) Solvent Saver Pressure Mode
Solvent Saver: PRESSURE
Pressure: 1500 psi
Temperature: 110 ° C
Heating time: 6 min
Static time: 15 min
Cycles: 1
Flush: 10 % (Recommended)
Purge: 120 sec
Total Time: 26-27 min
Solvent: Hexane

(C) Solvent Saver Flow Mode
Solvent Saver: FLOW
Pressure: 1500 psi
Temperature: 110° C
Heating time: 6 min
Static time: 15 min
Cycles: 1
Flush: 0 % (Default)
Purge: 120 sec
Total Time: 26-27min
Solvent: Hexane
Flow: 1 mL/min

NOTE ***Always use rinse before and after the extraction to make sure the lines are cleaned and no residue is left behind.***

NOTE ***The extraction conditions (such as static time, cycles and flush volume) can be tailored for a particular sample and extraction.***

2.6. Evaporation, drying and weighing:

Evaporate the collected solvent or extract in a water bath or any suitable heater equipped with a shaker (such as using a Dionex SE 500, module) at about 70 °C under constant nitrogen gas stream. Dry the collection bottle further in an oven at 75-100 °C for about 20-30 min to attain a constant weight of the bottle. The drying also ensures the removal of residual moisture present in the extract. Shake the bottle every 10 min for homogeneous drying. Remove the bottle from the oven and cool down the bottle to room temperature. Weight out the bottle and record this value. The difference between the bottle with the lipid sample and the empty bottle is the weight of the lipids recovered from the sample. Dry the bottle further for about 10 minutes, and cool down the bottle and re-weigh. Repeat drying if a constant weight is not achieved.

WARNING ***Ensure that the bottle reaches a constant weight otherwise erroneous results will be obtained.***

2.7. Base hydrolysis of food-procedure for GC/GS-MS determination:

Follow the procedure outlined in the AOAC method 996.06 for hydrolysis but use a 40 mL glass bottle instead of the mojonnier flask. Weigh out the food sample in a 40 mL vial. Adjust the amount of sample (between 0.1 and 0.5 g) so that approximately 100-200 mg of lipid is released during hydrolysis (AOAC 996.06). Add 0.1 g of pyrogallol (to prevent oxidative losses during hydrolysis). Add 2.0 mL of triglyceride internal standard solution to the vial. Add 2 mL of ethanol and mix the contents. Add 4 mL of Water/DI into the bottle and mix the contents. Finally add 2 mL of 58% NH_4OH (= 28-30% NH_3 content, CAS 1336-21-6), tighten/fix the cap and mix the contents thoroughly. The vortex can be used for mixing but with moderate agitation. Proceed with the hydrolysis step as described in section 2.3. Follow the procedure described in the section 2.4-2.6 for sample preparation, extraction and evaporation. Follow the esterification procedure according to AOAC method 996.06 for GC/GC-MS.

2.8. Cleaning the cell after extraction:

1. Remove the top end cap after extraction. Tap the cap on the lab bench. Squirt some solvent to remove any residual resin. Blow with pressurized lab air/N_2, if necessary.
2. Invert the cell on a waste (or collection) container and tap the cell body with 'filter insertion tool' slowly and the resin and DE will come out of the cell. Use a lab spatula or filter insertion tool to force out the residual mass.
3. Remove the bottom (other) end cap.
4. Remove all the residual mass plus filter from the cell.
5. Rinse the cell (inside) with water or deionized water thoroughly and dry it with paper towel. Blow with pressurized lab air/ N_2, if necessary.

SECTION 3 – TROUBLESHOOTING

Low Lipid Recovery

a. Check the sample. Generally older samples tend to give lower lipid recovery once they are opened and left under ambient conditions. Use a fresh container of the sample to check the lipid level. Check if reagents were added correctly.
b. Check the hydrolysis temperature and maintain it. The lipid recovery is affected by the low hydrolysis temperature.
c. Check for any instrument leaks.
d. Check the hydrolysis container for any sample loss to the wall and on the cap.
e. Check the evaporation temperature and ensure that is within recommended values. Applying higher temperatures can cause charring or brown coloration leading to lower recoveries.

High Lipid Recovery

a. Check the evaporation temperature. The solvent needs to be completely evaporated and dried in such a manner that the bottle has a near constant weight (and by default the sample). The bottle needs to cool down to room temperature before it is weighed.
b. Look for substantial water condensate in the collection bottle.
c. Check the moisture content of the DE (about 1%) and ASE Prep CR H+ form resin (about <20%). Dry the resin and DE in the oven, if necessary.

Discoloration of Filter

a. Ensure the bottom and top resin plugs are added in the cell.
b. Check the moisture content of the DE (about 1%) and ASE Prep CR H+ form resin (about <20%). Dry the resin and DE in the oven before use, if necessary.

Water in Extract

a. Check the sample preparation steps and ensure the top and bottom resin plugs are added in the cell.
b. Check the total liquid amount for base and ethanol during hydrolysis. More base and ethanol than specified amount may result in leaching out some water during extraction.
c. Check the moisture content of the DE (about 1%) and ASE Prep CR H+ form resin (about <20%). Dry the resin and DE in the oven, if necessary.
d. Dry the extract or sample for a longer period of time to ensure complete removal of any moisture present in the sample.

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