February 7, 2003

DRAFT Study Plan: Impurity Analysis of Articles

The following is a proposed study plan for analysis of PFOA in 11 articles treated by telomer-based products (see below). Samples will be collected from pilot plant facilities or will be collected from commercial manufacturing facilities. Analyses need to be completed by May 2003.

Types of Articles: representative Textile, Carpet and Paper articles (11 total)

The proposal is designed to determine whether PFOA is present in each article collected, and if it is present, at what levels and finally the confidence of those reported levels. This study, as proposed, is a *screening study*.

PROPOSED APPROACH

-An LC/MS/MS methodology will be used for these analyses having a lower limit of quantification of 1 ppb with a relative standard deviation of less than 20%.

-The study will be conducted as a GLP study at a contract lab.

-A preliminary extraction efficiency/method validation study will be conducted upfront

Preliminary Study

-A preliminary method validation focused on ensuring that extraction efficiency falls within 70-110% will be conducted for three matrices: carpet (nylon), paper and textile (polyester). Extraction efficiency for each matrix will be determined at three fortification levels (six replicates each):

1 ppb, 10 ppb and 100 ppb

Sample spiking should be conducted at an alkaline pH, to reduce losses of PFOA, especially at the 1 ppb level. If scouting experiments show that extraction efficiencies can be achieved readily within the 70-110% guideline, the contract lab will proceed with running additional sample sets on two separate days. The plan is to run 6 sample sets at each of the three spiking levels on each day for a total of 36 samples per matrix.

172 untreated articles will be required for this portion of the study (54 samples of untreated shaved carpet, 10 samples of carpet backing, 54 samples of textile and 54 samples of paper).

The results of this preliminary study will be prepared as a report, detailing the method specifics including:

Summary of method and results
Materials used
Equipment used
Reagents and standards used

Analytical Procedures
Preparation of stock standard solution, preparation of fortification of articles and preparation of calibration standards

Pre-processing of samples, sample fortification procedures, analyte extraction and final sample preparation procedures

Instrumentation
LC/MS conditions
LC/MS Calibration and Samples Analysis

Calculation Methods

Results and Discussion
Detector response
Control and/or Blank
Recoveries
Extraction efficiency (?)
Limit of Quantification and Limit of Detection
Time required for analysis
Special precautions

Conclusion

Article Analysis

Extraction efficiency for other articles:
Extraction efficiency will be determined and/or verified during sample analysis. Extraction efficiencies will be determined at 1 ppb, 10 ppb and 100 ppb. A control will be included in the analysis set.
Extraction / recoveries will be carried out at each of the three levels: 1 ppb, 10 ppb and 100 ppb (this may change depending upon results of the preliminary study).

Sampling Procedure:
Sampling is a critical aspect of analytical analysis that is often overlooked. If done right, and if the extraction efficiencies are good, then the study will produce results with high confidence. See separate Sampling Procedure.

Samples: Six samples analyzed for each article, selected using block design experiment.

SOP (standard operating procedure)/protocol for analysis of PFOA in test articles will be prepared and reviewed by analytical chemistry experts and possibly others before actual work begins. Work should begin on samples by mid April 2003.

Background for Method Development

Extraction Procedure: Methanol [Suggest that material be extracted with methanol at room temperature in closed-cap bottle using wrist-action shaker, followed by centrifuge at 3000 rpm]. Ratio of 4:1, methanol to article mass. Clean-up and concentrate using solid phase extraction procedure recommended by contract lab.

Sample: 10 grams each for carpet and carpet backing, 2 grams each for paper and textile
Sample Number: 6 sub-samples from each article
All prep done in plastic vials.
Sample should not be filtered unless particulates are observed.

Internal Standard: Internal standard isotopically enriched acid ($C_6F_{13}{}^{13}CF_2{}^{13}COOH$) can be supplied. Currently, the thinking is to add standard after extraction.

Standard Prep:
Stock Standards weighed in to plastic vial and solubilized in HPLC grade methanol.
Concentration of stock standard is 1000ppm (1mg/mL).
Serial dilutions made in Milli-Q Water.
Prepare five standards in the range of 0.1 ppb to 100 ppb - must bracket sample concentrations.

Suggested sequence for analysis:
Prepare at least four chromatographic standards of PFOA to bracket the levels expected in the fortifications and the samples to be analyzed. Each set of sample analyzed should include at least one control (untreated and unfortified sample), one control sample fortified at the limit of quantitation and one control sample fortified at the maximum level of quantitation. The control is used to demonstrate that baseline interference is less than 10% of the LOQ.

A control should be injected three times at the beginning of each automated sequence, followed by a standard. Standards should then be injected after every two to three samples, and the last injection of the sequence should be a standard. Standards and fortifications should be injected in order of increasing concentration.

If the concentration of either analyte in a set of sample exceeds the range of the standards, dilute with methanol.

No data is available on the stability of frozen samples.

When using the Linear Regression Method the coefficient of determination, r-squared, should be ≥ 0.99; whereas if the Response Factor Method is employed, the relative standard deviation of the standards analyzed for the sample set must be less than 20%.

LC/MS conditions based upon submitted procedures:

Product Analysis Method
LC Parameters
Instrument: Agilent 1100
Mobile Phase: A: 90% 2mM* Ammonium Acetate 10% Methanol
B: 100% Methanol
*prefer that the concentration of AA is bet. 10-20 mM

Gradient Profile

Time	%B	Flow
0	20	0.3mL/ min
12	100	0.3mL/min
15	20	0.3mL/min
18	20	0.3mL/min

Post Time: 2 min
Injection Volume: 25µL
Analytical Column: Spherisorb ODS 2.1 X 200 mm* 5 µm Packing
* prefer shorter column if resolution is good
Column Temperature 40°C

Mass Spec Parameters
Instrument: Micromass Quattro Ultima; Ion Mode: Electrospray Negative

Tuning Parameters
Source : Capillary 2.9 kV; Cone 10 V; Hexapole1 0.2 volts; Aperture1 0.0 volts; Source Block; Temp. 150°C; Desolvation Temp. 250°C
MS : Entrance 54 volts; Exit 75 volts; Ion Energy 0.6 volts; Ion energy ramp 0
MS2 : Ion energy 1.0 volt; Ion energy ramp 0; LMResolution 13.3; HMResolution 13.1; Multiplier 700 volts
Pressures : Gas Cell 9.0e-5; Cone gas 2.0e2 L/hr; Desolvation Gas 877 L/hr

Mass Spec MRM (multiple reaction monitoring)
MRM of 413 to 369

(details regarding similar approaches to the above are available on request)

Documentation

Letter summarizing results will be acceptable for May 2003 deadline. Final report will be required.