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**7.05 DETERMINATION OF MERCURY IN CERTIFIABLE COLOR  
ADDITIVES AND COLOR ADDITIVE LAKES BY AUTOMATED  
MICROWAVE DIGESTION AND DEDICATED MERCURY  
ANALYZER**

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**Section 1. Introduction**

21 CFR Parts 74 and 82 specify the maximum permitted levels of mercury in certifiable color additives and their lakes. In this method [1], a sample is mixed with a dilute solution of hydrochloric acid and nitric acid. Triton X-100 and/or ethanol are added as dispersing agents to those color additive samples, including lakes, that are not soluble in the acid solution. A portion of the sample solution or dispersion is digested by microwave heating under pressure, and the dye matrix is oxidized. Mercury is determined by atomic absorption spectrometry using a dedicated mercury analyzer. Calibration is performed with external standards.

Atomic absorption spectrometry using a dedicated mercury analyzer is similar to flame atomic absorption spectrometry, described in Method 7.03, except that mercury vapor is generated chemically with stannous chloride, rather than by atomization in a flame.

**Section 2. Apparatus**

- A. SpectroPrep Automatic Microwave Digestion System - CEM
- B. Mercury Analyzer - Thermo Separation Products
- C. Computer software
  - "FDA\_2mL" - SpectroPrep microwave digestion program, modified for color additives
  - "HGTALK" - Thermo Separation Products mercury analyzer program
  - "Mercury" - QuattroPro spreadsheet for calculating results, developed for this method
- D. Micropipet, 20-200  $\mu$ L - Gilson
- E. Analytical balance, accurate to 0.1 mg
- F. Top-loading balance, accurate to 1 mg
- G. Sample vessels, polypropylene, test tube shaped, 50 mL - Perkin Elmer, Cat. No. B019-3234

- H. **Sample vessels**, polypropylene, test tube shaped, 15 mL - Perkin Elmer, Cat. No. B019-3233
- I. **Mixer**, MaxiMix Plus, 120 V - Fisher Scientific, Cat. No. 12-815-18
- J. **Vials**, screw cap, glass, 6 dram - Kimble, Cat. No. 60910-L
- K. **Test tubes**, glass, 100 × 13 mm
- L. **Regulator for nitrogen**

### Section 3. Reagents

- A. **Ethanol**, 95%, USP grade
- B. **Hydrochloric acid**, for trace metal analysis, "Instra-analyzed" - Baker, Cat. No. 9530-00
- C. **Magnesium perchlorate**, reagent grade
- D. **Nitric acid**, for trace metal analysis, "Instra-analyzed" - Baker, Cat. No. 9598-00
- E. **Sodium chloride**, reagent grade
- F. **Stannous chloride**, anhydrous, reagent grade
- G. **Sulfuric acid**, for trace metal analysis, "Instra-analyzed" - Baker, Cat. No. 9673-00
- H. **Triton X-100**, reagent grade
- I. **Water**, deionized, < 50 ppt mercury
- J. **Nitrogen**, ultra-high purity
- K. **Standard**

Mercury atomic absorption standard reference solution, 10.00 mg/mL (10,000 ppm) - National Institutes of Standards and Technology, Standard Reference Material 3133

### Section 4. Preparation of Solutions

#### A. 8% Hydrochloric acid + 24% nitric acid

Add ca 1000 mL of water to a 2000 mL volumetric flask. Using graduated cylinders, add 160 mL of hydrochloric acid and 480 mL of nitric acid. Swirl to mix and allow to cool to room temperature.

Dilute to volume with water, stopper, and shake to mix. Use until consumed.

**B. 4% Hydrochloric acid + 12% nitric acid**

Add ca 1000 mL of water to a 2000 mL volumetric flask. Using graduated cylinders, add 80 mL of hydrochloric acid and 240 mL of nitric acid. Swirl to mix and allow to cool to room temperature. Dilute to volume with water, stopper, and shake to mix. Use until consumed.

**C. 1% Hydrochloric acid + 3% nitric acid**

Using a graduated cylinder, dilute 100 mL of the 4% hydrochloric acid + 12% nitric acid solution to 400 mL with water. Transfer to a 500 mL volumetric flask, stopper, and shake to mix. Use until consumed.

**D. 5% Sulfuric acid**

Add ca 1000 mL of water to a 2000 mL volumetric flask. Using a graduated cylinder, add 100 mL of sulfuric acid. Swirl to mix and allow to cool to room temperature. Dilute to volume with water, stopper, and shake to mix. Use until consumed.

**E. 2% Stannous chloride**

Using top-loading balance, add 20 g of anhydrous stannous chloride and 30 g of sodium chloride to a 1000 mL volumetric flask. Slowly and carefully add 50 mL of sulfuric acid. Heat and gases will evolve. Add 10 to 20 mL of water to rinse down the sides of the flask; do not add more water because the solids will not dissolve. Swirl until the solids dissolve. Allow to cool to room temperature. Dilute to volume with water, stopper, and shake to mix. Prepare monthly.

**Section 5. Standard Solutions****A. Mercury, 100 ppm (100 ppm Hg)**

Pipet 1 mL of the standard mercury solution into a 100 mL volumetric flask. Dilute to volume with the 4% hydrochloric acid + 12% nitric acid solution, stopper, and shake to mix. Prepare yearly.

**B. Mercury, 100 ppb (100 ppb Hg)**

Using a micropipet, add 50  $\mu$ L of the 100 ppm Hg solution to a 50 mL volumetric flask. Dilute to volume with the 4% hydrochloric acid + 12% nitric acid solution, stopper, and shake to mix. Prepare daily.

**C. 0.2 ppb Hg check standard**

Using a micropipet, add 100  $\mu$ L of the 100 ppb Hg solution to a 50 mL volumetric flask. Dilute to volume with the 1% hydrochloric acid + 3% nitric acid solution, stopper, and shake to mix. Prepare daily.

**Section 6. Procedure****A. Preparation of samples and blank for microwave digestion**

Do not analyze FD&C Red No. 3, D&C Orange No. 10, and D&C Orange No. 11 samples. Those color additives contain iodine, which can penetrate Teflon components and bind mercury.

Using an analytical balance, weigh 0.030 g ( $\pm$  0.005 g) of each sample into a 50 mL polypropylene tube, and record the weight to the nearest 0.1 mg. Prepare one spiked sample for every 10 unknown samples by pipetting 300  $\mu$ L of the 100 ppb Hg solution into the tube. Prepare a digestion blank by adding 50 mL of 20% nitric acid solution to a 50 mL polypropylene tube.

- (a) **Samples of FD&C Blue No. 1, FD&C Blue No. 2, FD&C Green No. 3, FD&C Red No. 40, FD&C Yellow No. 5, FD&C Yellow No. 6, D&C Green No. 5, D&C Green No. 8, D&C Orange No. 5, D&C Yellow No. 8, D&C Yellow No. 10, Ext. D&C Yellow No. 7, and their lakes**

To each sample, add 50 mL of 4% hydrochloric acid + 12% nitric acid solution. Cap each tube and shake until the sample is completely dissolved.

- (b) **Samples of Citrus Red No. 2, D&C Green No. 6, D&C Orange No. 4, D&C Red No. 6, D&C Red No. 7, D&C Red No. 17, D&C Red No. 21, D&C Red No. 22, D&C Red No. 27, D&C Red No. 28, D&C Red No. 30, D&C Red No. 33, D&C Red No. 34, D&C Red No. 36, D&C Violet No. 2, and their lakes**

To each sample, add 3-5 drops of 95% ethanol and swirl to mix. Add 25 mL of warm water and swirl to mix. Add 25 mL of 8% hydrochloric acid + 24% nitric acid solution and 1 drop of Triton X-100. Cap the tube and shake until the sample is completely dispersed.

- (c) **FD&C Red No. 4 samples**

To each sample, add 50 mL of 4% hydrochloric acid + 12% nitric acid solution and 5 drops of Triton X-100. Cap the tube and shake until the sample is completely dispersed.

#### **B. Microwave digestion**

Uncap the sample tubes. Load the samples and blank into the autosampler of the SpectroPrep. Place labeled 15 mL polypropylene collection tubes in their holders. Turn on the SpectroPrep computer. Load the program FDA\_2mL and start it. At the end of the digestion run, record the volume ( $\pm 0.1$  mL) of each digested solution relative to the marking on the collection tube.

#### **C. Preparation of calibration solutions for atomic absorption analysis**

The mercury analyzer is quantitatively calibrated with blanks and with five calibration solutions whenever new 2% stannous chloride and 5% sulfuric acid solutions are prepared. Calibration is performed in the absence of added color additive. Between calibrations, the 0.2 ppb Hg check standard solution is analyzed.

Table 7.05/1 gives mercury concentrations in each calibration solution. Prepare the calibration solutions as follows: Micropipet aliquots of the 100 ppb Hg solution into each of five 50 mL volumetric flasks. Dilute the solutions to volume with 1% hydrochloric acid + 3% nitric acid solution, stopper, and shake to mix. Place portions of each solution in labeled 6-dram glass vials.

Table 7.05/1 Mercury concentrations in calibration solutions

Calibration Solution	Aliquot of 100 ppb Hg Solution ( $\mu\text{L}$ )	Concentration of Mercury (ppb)
1	0	blank
2	100	0.2
3	200	0.4
4	300	0.6
5	400	0.8
6	500	1.0

#### D. Determination

During the sample digestions, turn on the mercury analyzer. Be sure that the reagent bottles are filled with 5% sulfuric acid and 2% stannous chloride solutions, and empty the waste container. Unscrew the drying tube from its fitting while holding the fitting stationary. Replace the contents of the drying tube with fresh magnesium perchlorate. Replace the drying tube. Be careful not to crimp the tubing near the fitting.

Place the calibration blank and the check standard in autosampler positions 1/1 and 1/2. Place the remaining calibration solutions in the subsequent positions. Remove the collection tubes from the SpectroPrep, cap, and shake to mix. Transfer to labeled test tubes. Place the digestion blank in position 2/1, and place the sample solutions in the subsequent positions.

Load the HGTALK software. Recall and run the program BLANK&ST.HSQ to ensure a stable baseline and clean reaction cell. Repeat until satisfied. If the result for the check standard deviates by more than  $0.2 \text{ ppb} \pm 0.03 \text{ ppb}$ , check its preparation or recalibrate the instrument with fresh calibration solutions.

Recall a recent sequence file and edit as follows: Ensure that the method file is C:\HGTALK\METHOD\FIFTH.HMH. Change the filename for the data file using the new date and analytical run. (For example, C:\HGTALK\DATA\95080601 corresponds to the first run on August 6, 1995.) Enter the calibration solutions and samples to be analyzed in the sequence file. After every 10th sample, enter a spiked sample, a check standard, and a calibration blank. Save the sequence file as C:\HGTALK\SEQUENCE\95080601.HSQ (using the appropriate date and run). Load and run the sequence file.

### Section 7. Calculations

Enter the following into the QuattroPro spreadsheet "Mercury": the data for Hg found in ppb (from the mercury analysis print-out), the sample weights, and the digestion volumes. An example is shown in Table 7.05/2. Obtain the results from the spreadsheet program. If the result for a spike recovery or check standard deviates from 100% by more than 20%, determine and correct the cause and reanalyze affected samples. Record the results for ppm Hg in each sample in the appropriate notebook. The error of the method for 1 ppm Hg is estimated to be 0.3 ppm at the 95% confidence level.

Table 7.05/2 Example of Mercury spreadsheet

A ID or Top number	B ppm Hg added	C Sample wt, g	D $\mu$ L 100 ppb Hg	E Digest. vol., mL	F Dil. factor	G Hg found, ppb	H Found less blank, ppb	I Hg in sample, ppm	J Spike recov., %	K Normaliz. factor
BLANK	0	0	0	10.3	3.96	0.0277	0.0000	0.0000	ERR	0.002689
963164	0	0.0316	0	10.4	7.27	0.0304	0.0024	0.0177	ERR	
963173	0	0.0273	0	10.2	7.19	0.0111	-0.0163	-0.1173	ERR	
963173	1	0.0252	300	10.2	7.78	0.1619	0.1345	1.0467	88	

where

- A = sample identification or top number
- B = ppm Hg added to spiked sample
- C = sample weight (g)

- D** =  $\mu\text{L}$  of 100 ppb Hg solution added to spiked sample
- E<sub>b</sub>** = digestion volume of blank (mL)
- E<sub>s</sub>** = digestion volume of sample (mL)
- F** = dilution factor for sample (ppm/ppb)  
=  $(50 \text{ mL} \times 1 \text{ g/mL} \times E_s \times 10^{-3} \text{ ppm/ppb}) / (C \times 2.6 \text{ mL})$
- G<sub>b</sub>** = Hg found in blank solution (ppb) (instrument reading)
- G<sub>s</sub>** = Hg found in sample solution (ppb) (instrument reading)
- H** = Hg found less blank (ppb)  
=  $G_s - (K \times E_s)$
- I** = Hg in sample (ppm) (calculated using dilution factor)  
=  $H \times F$
- J** = spike recovery (%)  
=  $H \times F \times C \times 10^6 / D$
- K** = normalization factor for blank vs sample (ppb/mL)  
=  $G_b / E_b$

## REFERENCE

1. Hepp, N. Manuscript in preparation.