AOAC Official Method 997.15
Lead in Sugars and Syrups
Graphite Furnace Atomic Absorption Method
First Action 1997

A. Principle

Test portions are wet ashed with HNO_3–H_2O_2 and analyzed using graphite furnace atomic absorption spectrophotometry (GFAAS). Magnesium nitrate is used as a matrix modifier, with air ashing, platform atomization, and quantitation by direct calibration against aqueous standards using peak area measurements at 283.3 nm. Care must be taken to avoid contamination. *See* Table **997.15** for results of interlaboratory study.

B. Apparatus

(**a**) *Atomic absorption spectrophotometer*.—Graphite furnace atomic absorption spectrophotometer set at 283.3 nm with autosampler, pyrolytically coated tubes, solid pyrolytic graphite platforms, and adequate means of background correction required. Zeeman effect or Smith-Hieftje background preferred, but deuterium arc background correction acceptable. [The method was developed on a Perkin-Elmer Model Z5100 (0.7 nm slit, HGA600 furnace, AS-60 autosampler) with Zeeman background correction.] If instrument does not have well defined calibration function, separate calculator or computer is required for linear least squares, nonlinear, or quadratic calibrations. Use either hollow cathode lamp or electrodeless discharge lamp as source and Ar as purge gas and breathing quality air (for air ashing to avoid C build up during char step) as alternative gas. Set up instrument according to manufacturer's specifications with consideration of good GFAAS practice, addressing such factors as line voltage, cooling water temperature, graphite part specifications, and furnace temperature. If optical pyrometer or thermocouple is not available to check furnace controller temperature calibration, dim room lights and observe the furnace emission through introduction port while increasing furnace temperature. Characteristic cherry red glow should begin to appear at 800°C. If furnace glows at a lower indicated temperature (typical with longitudinal Zeeman furnaces) then furnace is hotter than indicated temperature and temperature settings must be adjusted downwards accordingly.

(**b**) *Plasticware and glassware*.—Clean Teflon or polyethylene autosampler cups in mixture of 5% HNO_3 + 5% HCl made up in deionized distilled water (18 MΩ·cm), and thoroughly rinse with deionized distilled water to avoid contamination. Use micropipets with disposable lead-free tips for dilution. Assure volumetric accuracy and precision of micropipets and tips by dispensing and weighing 5–10 replicate portions of water onto microbalance. Acceptable accuracy is 98–102% recovery. Use acid-cleaned volumetric glassware (10 and 100 mL) to prepare standards and to dilute test solutions to final volume. For digestion, use 15–20 mL acid-cleaned high density polyethylene tubes, polypropylene tubes, Teflon tubes, or quartz tubes. Store final diluted test solutions in acid-cleaned capped plastic tubes.

C. Reagents

(**a**) *Nitric acid*.—High purity or sub-boiling distilled, certified to contain ≤0.04 μg Pb.

(**b**) *5% Nitric acid*.—5% (v/v) prepared from high purity HNO_3, (**a**), and 18 MΩ·cm water.

(**c**) *Hydrogen peroxide*.—50% H_2O_2.

(**d**) *Modifier stock solution*.—Weigh 20 g of ultrapure (99.99%) $Mg(NO_3)\cdot 6H_2O$ and dilute to 100 mL with 18 MΩ·cm water. Just prior to use, prepare modifier working solution by diluting the stock solution 1:10. A volume of 5 μL will provide 0.06 mg $Mg(NO_3)_2$.

(**e**) *Standard solutions*.—(*1*) *Daily lead stock solution*.—1 μg/mL. Use a high purity single element 1000 or 10,000 μg/gL Pb stock solution to prepare an intermediate 10 μg/mL Pb stock solution weekly in 5% HNO_3 (**b**). Suitable standards are available from commercial sources and from NIST, Gaithersburg, MD 20899 USA, as SRM 3128, 10,000 μg/mL Pb. Prepare daily a 1 μg/mL Pb solution by pipetting 10 mL of the intermediate 10 μg/mL Pb solution into a 100 mL volumetric flask and diluting to volume with 5% HNO_3, (**b**).

(*2*) *Daily working calibration standards*.—Prepare daily working calibration standards of 10.0, 25.0, 50.0, and 100 ng/mL from 1 μg/mL daily lead stock solution, **C**(**e**)(*1*), by pipetting 100, 250, and 500 μL, and 1 mL aliquots into individual 10 mL volumetric flasks and diluting to volume with 5% HNO_3. If GFAAS autosampler is used to automatically dilute standards, assure calibration accuracy by pipetting volumes of ≥3 μL.

(**f**) *Argon*.—High purity (≥99.9%).

(**g**) *Hydrochloric acid*.—High purity or sub-boiling distilled certified to contain ≤0.005 μg/L Pb.

D. Digestion

(*Caution*: Perform procedure in fume hood and wear safety glasses.)

Obtain representative test sample for analysis. For liquids such as sugar syrups, ultrasonicate and/or Vortex mix prior to weighing. For solids such as crystalline sucrose, make sugar solution using equal weights of sugar (5 g minimum) and 18 MΩ·cm water. Mix until completely dissolved. Accurately weigh 1.5 g to nearest 0.001 g of liquid (or 3.0 g of sugar solution equivalent to 1.5 g of solids) into digestion tube. Run preparation blank of 1.5 g of 18 MΩ·cm water through entire procedure with each batch of tests. Add 0.75 mL nitric acid. Heat plastic tubes in water bath, quartz tubes in water bath or heating block, warming slowly from 30° to 95°C over about 30 min (to avoid spattering). Monitor temperature of "dummy" tube by placing glass thermometer in the tube. Heat until all brown vapors have dissipated and

Table 997.15 Results of interlaboratory study for determination of lead in sugars and syrups (100 ng/g level)

Material	No. of labs	s_r	s_R	RSD_r, %	RSD_R, %	r^a	R^b
Sucrose	8	2.67	14.0	2.67	14.0	7.48	39.2
Fructose	8	6.53	19.0	6.53	19.0	18.3	53.2

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

any rust colored tint is gone (20–30 min). Cool. Add 0.5 mL 50% H_2O_2 dropwise and heat at 90–95°C for 5 min. Cool. Add second 0.5 mL portion 50% H_2O_2 dropwise and heat at 90–100°C for 5–10 min until clear. Cool and dilute to final volume of 10 mL.

E. Determination

(**a**) *GFAAS start-up*.—Switch on spectrometer and follow manufacturer's instructions to adjust lamp current, slit, and wavelength (283.3 nm). Insert platform into graphite tube, position tube in atomizer head, and set required program for furnace as follows: (*1*) Dry at 200°C using 20-s ramp, 30-s hold, and 300 mL/min Ar flow; (*2*) char the test solution at 750°C using 40-s ramp, 40-s hold, and 300 mL/min air flow; (*3*) cool down and purge air from furnace with Ar for 60-s using 20°C set temperature and 300 mL/min Ar flow; (*4*) atomize at 1800°C using 0-s ramp and 10-s hold with Ar flow stopped; (*5*) clean out at 2600°C with 1-s ramp and 7-s hold; (*6*) cool down furnace (if necessary) to 20°C with 1-s ramp and 5-s hold with 300 mL/min Ar flow.

Use autosampler to inject 20 μL of blanks, calibration standards, and test solutions, and 5 μL modifier working solution. Analyze each test solution in triplicate and average integrated absorbance (peak area) results. After ensuring that furnace is clean by running 5% HNO_3 blank, check instrument sensitivity by running 25 ng/mL calibration standard. If integrated absorbance is <0.14 abs-s for standard 28 × 6 mm end-heated furnace tube, or <0.05 abs-s for side-heated longitudinal furnace tube, determine cause of the reduced sensitivity and correct as appropriate. If integrated absorbance is >0.25 abs-s, contamination is likely and source should be investigated. Calculate characteristic mass (m_o) which is mass of lead in pg necessary to produce integrated absorbance of 0.0044 abs-s as follows:

$$m_o = \frac{(0.0044 \text{ abs} - \text{s})(25 \text{ pg}/\mu\text{L})(20\mu\text{L})}{\text{measured } 25 \text{ pg}/\mu\text{L soln. abs} - \text{s}}$$

Record and track integrated absorbance and m_o for reference and quality assurance.

(**b**) *Calibration standards*.—Inject each calibration standard in triplicate starting with lowest concentration. Typical instrument linearity extends to 25 ng/mL with 20 μL injections. If nonlinear calibration capability (e.g., rational, quadratic) is not available, or does not provide a good fit, limit working calibration curve to ≤25 ng/mL. Use calibration algorithms provided in the instrument software if available. Recheck calibration periodically (≤15 test portions) by running 25 or 50 ng/mL calibration standard interspersed with tests. If recheck differs from calibration by >10%, recalibrate instrument. The instrumental detection limit (DL) and quantitation limit (QL) in picograms, may be based on 10 replicates of preparation blank and calculated as follows: DL = 3 times the standard deviation of blank; QL = 10 times standard deviation of blank. Calculate mass of lead in blank in pg as follows:

$$Pb_b = (A_{blank}/A_{std})(\text{Std})(20\ \mu\text{L})$$

where A_{blank} is the integrated absorbance for the blank, A_{std} is the integrated absorbance for the standard, and Std is the concentration of the standard in pg/μL.

Detection limits are typically 10–14 pg corresponding to 0.5–0.7 ng/mL for 20 μL injections, this corresponding to method detection limit of 3.3–4.7 ng/g sugar.

(**c**) *Quality assurance*.—To assure analytical accuracy, anaylyze NIST SRM 1643c acidified water or similar material directly (without digestion) prior to unknowns (using specified analyte analysis method shown below). The certified content of SRM 1643c is 35.3 ± 0.9 ng/mL. If concentration determined is not within 10% of mean reference value (31.8–38.8 ng/mL), reason for inaccuracy must be evaluated and unknowns should not be analyzed until acceptable accuracy is achieved. Prepare an in-house control from uncontaminated table sugar or reagent grade sucrose (or other appropriate substance with Pb content <5 ng/g as received) and mix with equal volume of water. Spike this solution with Pb to produce a concentration of 100 ng/g. Digest and analyze with each batch analyzed. Recoveries should be 100 ± 20% and precision for complete replicate digestions should be <10% RSD. Periodically, check a sample digest using method of standard additions to ensure no multiplicative or chemical interferences exist.

(**d**) *Analysis*.—Analyze each test solution in triplicate and record integrated absorbances. If instrument response exceeds that of calibration curve, dilute with 5% HNO_3 to bring analyte response into working range and note dilution factor (DF). Dilute test solutions which have a final concentration >25 ng/mL 1:10 to facilitate analysis in linear range for systems not equipped with nonlinear calibration algorithms. Correct all analyses using previous preparation blank. This can typically be automatically done by instrument software after identifying and running a representative preparation blank. Use calibration algorithm provided in instrument software to calculate blank corrected Pb concentration (ng/mL) based on integrated absorbance measurements. Final concentration of a test solution is based on the average of the 3 replicate test solutions analyzed.

F. Calculations

Lead level present in the original sugar or syrup test samples are calculated as follows:

$$\text{Pb (ng/g)} = \frac{(\text{blank corrected Pb ng}/\text{mL})(\text{DF})(\text{test solution vol})}{(\text{test portion weight})}$$

where DF is the dilution factor.

References: *J. AOAC Int.* **77**, 1288(1994).
J. Ag. Food Chem. **43**, 923(1995).

* If sugar solution was prepared initially to assure homogeneity, test portion weight is weight of original sugar digested (not weight of solution).