Method for Total Selenium – Selenized Yeast
ME SOP 85 and ME SOP 42b

1.0 Foreword

1.1 The method given is a guide for sample preparation and/or digestion with minerals/metals analysis by ICP or ICP/MS including the setup, calibration, and instrument quality control involved in the analysis of sample preparations by EPA Method 6010B.[1]

2.0 Introduction

3.0 Title: Digestion Produceure for Yeast Samples: ME SOP 85 and Analysis of Solids/ Sludges by ICAP Spect: ME SOP 42b

4.0 Warnings

4.1 Inorganic acids are highly corrosive and must be handled carefully. All acid additions are carried out in the hood and technicians are to wear protective eyewear and face shield when working with the acid. The sample extractions are also carried out in a hood to minimize release of acid fumes into laboratory air.

5.0 Scope

5.1 Applicable to selenium enriched yeast in the range of 50- 3000 ppm.
5.2 For sample preparation/extraction of selenium. Other metals and mineral may be applicable.
5.3 Inductively coupled plasma-atomic emission spectrometry (ICP-AES) determines trace elements, including metals, in solution. Matrices included in this method include but not limited to: ground water, soil, sludge, sediments, solid wastes, TCLP extractions, and aqueous samples. Each matrix requires suitable sample preparation prior to analysis.

6.0 Normative references

6.1 Methods of analysis
6.1.1 Digestion Procedure for Yeast Samples
6.1.1.1 AOAC 969.32 [2]
6.1.1.2 AOAC 985.01 [2]
6.1.1.3 EPA 6010 [1]
6.1.2 Analysis of Solids/Sludges by ICAP-AES (6010B). [1]

7.0 Definitions

7.1 ICP: Inductively Coupled Plasma
7.2 ICP/MS: Inductively Coupled Plasma/Mass Spectrometer
7.3 ICP-AES: Inductively Coupled Plasma-Atomic Emission Spectrometry
7.4 HCl: Hydrochloric Acid
7.5 HNO_3: Nitric Acid
7.6 g: grams
7.7 ml: milliliters
7.8 D.I: Deionized
7.9 LCS: Laboratory Control Sample
7.10 RPD: Relative Percent Difference
7.11 ICV: Initial calibration verification
7.12 CCV: Continuing calibration verification
7.13 ICB: Initial calibration blank
7.14 CCB: Continuing calibration blank

8.0 Principle

8.1 Homogenized samples are digested so as to destroy the sample matrix and solubilize the elements of interest. Various sample types, many of unknown origin, are treated with hydrochloric, nitric acid and heat. The quantity of sample is varied to accommodate analysis of high levels of analytes, representative sub-sampling and effective digestion. Acid levels and heat are adjusted to effect completion of digestion as evidenced by a clear solution and no solids at the bottom of the sample extract. The method measures element levels by use of characteristic emission spectra. Aerosol of sample preparations is created by a nebulizer and transported by the nebulizing gas to radio frequency argon plasma. The element specific spectra are produced by interaction of the plasma with the sample. The spectra of the sample are separated by a grating spectrometer that provides the line spectra to photon detection devices. The intensity of individual wavelengths and their surrounding spectra are determined. Background levels are removed allowing calculation of the specific wavelength intensity which is proportioned to the amount of emitting element present.

9.0 Reactions

9.1 Not applicable

10.0 Reagents and materials

10.1 Reagent or trace metals grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the

accuracy of the determination. If the purity of a reagent is in question analyze for contamination. If the concentration of the contamination is less than the MDL then the reagent is acceptable.

10.1 Hydrochloric acid (Concentrated HCl)
10.2 Nitric acid (Concentrated HNO3)
10.3 Reagent water: All references to water in the method refer to reagent water unless otherwise specified. Reagent water will be interference free.
10.4 Applicable LCS material
10.5 Standard Stock Solutions may be purchased or prepared from ultra-high purity grade chemicals or metals (99.99% pure or greater). All salts must be dried for 1 hour at 105°C, unless otherwise specified.
10.6 Three types of blanks are required for the analysis for samples prepared by any method other than3020. The calibration blank is used in establishing the analytical curve, and the method blank is used to identify possible contamination resulting from varying amounts of the acids used in the sample processing.
10.7 The calibration blank is reagent water.
10.8 The continuing calibration blank is prepared by acidifying reagent water to the same concentration of the acids found in the standards and samples.
10.9 The method blank must contain all of the reagents in the same volumes as used in the processing of the samples. The method blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.
10.10 The Initial Calibration Verification (ICV) is prepared by the analyst by combining compatible elements from a dtandard source different than that of the calibration standard and at concentrations within the linear working range of the instrument.
10.11 The Continuing Calibration Verification (CCV) should be prepared in the same acid matrix using the same standards used for calibration at a concentration near the mid-point of the calibration curve. It is the same as the ICV.
10.12 The Interference Check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors.

11.0 Apparatus

11.1 Hot plate or hot block
11.2 Beakers
11.3 Volumetric flasks (of suitable precision and accuracy)
11.4 Volumetric pipettes (of suitable precision and accuracy)
11.5 Filter paper
11.6 Funnels
11.7 Watch glass
11.8 Thermo Jarrell Ash 61E ICP
11.9 Argon gas supply (high purity)

12.0 Sampling

12.1 Sample preservation and storage is variable based upon nature of samples. In general, no preservation is needed, but store in a cool refrigerator at 4°C.

12.2 Aqueous samples are preserved with nitric acid. The holding time for the sample is six (6) months.

13.0 Procedure

13.1 Weigh 1.0 grams of well mixed, homogenized sample to a 300 ml beaker. Record weight in notebook.

13.2 Carefully add 10 ml of concentrated nitric acid and 10 ml of concentrated hydrochloric acid. This is carried out in the hood.

13.3 Cover with watch glasses and heat at 75°C for 10 minutes on a hot plate. Increase temperature to 150°C for two (2) hours.

13.4 Cool to room temperature and quantitatively transfer to 100 ml volumetric flasks. Bring to volume with D.I. water. Mix well by inverting at least four (4) times.

13.5 Set up the ICP-AES instrument with proper operating parameters established as detailed below. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 minutes of operation prior to calibration). Operating conditions – The analyst should follow the instructions provided by the instrument manufacturer.

13.6 Before using this procedure to analyze samples, there must be data available documenting initial demonstration of performance. The required data document the selection criteria of background correction points; analytical dynamic ranges, the applicable equations, and the upper limits of those ranges; the method and instrument detection limits; and the determination and verification of inter-element correction equations or other routines for correcting spectral interferences. This data must be generated using the same instrument, operating conditions and calibration routine to be used for sample analysis. These documented data must be kept on file and be available for review by the data user or auditor.

13.7 Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on each particular instrument. All measurements must be within the instrument linear range where the correction equations are valid.

13.8 Method detection limits must be established for all wavelengths utilized for each type of matrix commonly analyzed. The matrix used for the MDL calculation must contain analytes of known concentrations within 3-5 times the anticipated detection limit.

13.9 The upper limit of the linear dynamic range must be established for each wavelength utilized by determining the signal responses from a minimum of three, preferably five, different concentration standards across the range. One of these should be near the upper limit of the range. The ranges which may be used for the analysis of samples should be judged by the analyst from the resulting data. The data, calculations and rationale for the choice of range made should be documented and kept on file. The upper range limit should be an observed signal no more than 10% below the level extrapolated from lower standards. Determined analyte concentrations that are above the upper range limit must

be diluted and reanalyzed. The analyst should also be aware that if an inter-element correction from an analyte above the linear range exists, a second analyte where the inter-element correction has been applied may be inaccurately reported. New dynamic ranges should be determined whenever there is a significant change in instrument response. For those analytes that periodically approach the upper limit, the range should be checked every six months. For those analytes that are known interferences, and are present at above the linear range, the analyst should ensure that the inter-element correction has not been inaccurately applied.

13.10 Profile and calibrate the instrument according to the instrument manufacturers recommended procedures. Flush the system with the rinse between each standard. (Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error). The calibration curve consists of a blank and five standards. Below is a list of the standards and their associated elements. They are purchased from an outside manufacturer of standards and the diluted.

13.10.1

Standards	**Elements**
Std blank	None
ICAL 1	Ca(high), Mg (high), K, Na, Ni, Zn (low), Mn, Ag, Cr
ICAL 2	Al, Ba, Fe(low), Co, V, Cu, Be
ICAL 3	As, Ti, Cd, Pb, Se
ICAL 4	Fe(high), Mn, Zn(high)
XAAL12	Fe(high), Mn, Zn(high), B, Mo, S, Si, Sn, P, Ti, Sr

13.11 For all analytes and determinations, three ICVs and a calibration blank must be analyzed immediately following daily calibration. A calibration blank and three continuing calibration verifications (CCVs) must be analyzed after every tenth sample and at the end of the sample run. Below is a list of the names of the continuing calibration blank and the three ICV/CCVs and their associated element levels. They are purchased from an outside manufacturer of standards and the diluted.

ICV's	**Elements**
QC Blank	None
ICAP19	1 ppm – As, Be, Cd, Ca, Cr, Co, Cu, Fe, Mn, Mn, Mo, Ni, Pb, Sb, Se, Tl, Ti, V, Zn
Low ICAP 19	0.05 ppm – As, Be, Cd, Ca, Cr, Co, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Tl, Ti, V, Zn
ICAP7	1 ppm – Al, Ba, B, Ag, Na 10 ppm – K 0.5 ppm – Si

Analysis of the ICV's must verify that the instrument is within ±10% of calibration with relative standard deviation <5% from replicate (minimum of two) integrations. If the calibration cannot be verified within the specified limits, the sample analysis must be discontinued, the cause determined and the instrument recalibrated. All samples following the last acceptable ICV/CCV must be reanalyzed. The analysis data of the calibration blank, and ICV or CCV must be kept on file with the sample analysis data

13.12 Rinse the system with a rinse of reagent water

14.0 Calculation

14.1 There are no calculations carried out in the digestion procedure; however for production of future dilution factors, the sample weight and final volume values are very important.
14.2 During anlysis, if dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported with up to three significant figures.
14.3 Sample weights, volumes, final volumes and dilution factors are entered upon above creation of the autosampler sequence.

15.0 Precision

15.1 Method Performance Criteria:

15.1.1 Visual observation of the sample should be carried out to ascertain if complete digestion occurred.
15.1.2 Make sure the laboratory number are correctly transcribed from the sample to the data table to the sample extract vial.
15.1.3 Record all times of extract, sample amount and final volumes.
15.1.4 ICB and CCB levels must be within two (2) times the determined IDL.
15.1.5 ICV levels must be within 5% of the true value.
15.1.6 LLICV levels must be within two (2) times the IDL. If the true level is over 10 times the IDL, the levels must be within 20% of the true level.
15.1.7 CCV levels must be within 10% of the true value.
15.1.8 ICSA non interfereant levels are to be less than three (3) times the IDL. Interferant levels are to be within 20%.
15.1.9 ICSAB: non interferant levels are to be within 10% of the three value or within two (2) times the IDL of the true value.

16.0 Quality assurance and control

16.1 Digestion

16.1.1 Quality Control Requirements: Prepare/digest one blank, at least one (1) LCS, if available, a replicate per batch, and a replicate per matrix type. In general, it is advisable to batch significantly different matrices into their own preparation batch.
16.1.2 Calibrate the balance prior to each batch preparation. Use of two 92) certified weights that bracket the sample amounts are used.
16.1.3 Do routine checks of the hot plate to demonstrate the required temperature is achieved and that there are no hot spots or cool spots

16.2 Analysis

16.2.1 The following are analyzed immediately after calibration: ICB, ICV's (ICAP 19, X17, ICAP7), LLICV, ICSA, ICSAB.

16.2.2 After every ten samples and at the end of the analytical run, the following are analyzed: CCB, ICV;s, (ICAP 19, X17, ICAP 7), ICSA, ICSAB.

16.3 Out of Control Data

16.3.1 ICB, ICU, LLICV, ICSA, ICSAB failure at start of run require diagnosis of problem and recalibration or reanalysis of the failing entity, prior to sample run start.

16.3.2 CCV or CCB failure(s) require reanalysis of samples back to the last acceptable CCV or CCB of that entity.

17.0 Special cases

17.1 Digestion Interferences

17.1.1 Variable based upon nature of samples. In general, possible matrix or spectral. Evaluate on individual sample matrix. Matrix interferences are generally to be managed by this preparation procedure in the destruction of the material. Future spectral interferences may be encountered in the analysis if certain elements are high enough to create overlaps at the wavelength or mass of interest. Some sample types may increase in volume to the point of escaping the beaker.

17.2 Analytical Interferences

17.2.3 Special interferences are caused by background emission from continuous or recombination phenomena, stray light from the line emission of high concentration elements, overlap of a spectral line from another element or unresolved overlap of molecular band spectra.

17.2.4 Spectral overlaps can be compensated by equations that correct for inter-element contributions. The interfering elements must be analyzed at the same time as the element of interest. When operative and uncorrected, interferences will produce false positive determinations and be reported as analyte concentrations. More extensive information on interferent effects at various wavelengths and resolutions is available in reference wavelength tables and books. Users may apply inter-element correction equations determined on their instruments with tested concentration ranges to compensate (off line or on line) for the effects of interfering elements.

17.2.5 The interference effects must be evaluated for each individual instrument. For each instrument, intensities will vary not only with optical resolution but also with operating conditions (such as power, viewing height and argon flow rate). The analyst is required to determine and document for each wavelength the effect from referenced interferences. The analyst is encouraged to utilize a computer routine for automatic correction on all analyses.

17.2.6 If the correction routine is operating properly, the determined apparent analyte(s) concentration from analysis of each interference solution should fall within a specific concentration range around the calibration blank. The interference check solutions should be analyzed more than once to confirm a change has occurred. Adequate rinse time between solutions and before analysis of the calibration blank will assist in the confirmation.

17.2.7 When inter-element corrections are applied, their accuracy should be verified, daily, by analyzing spectral interference check solutions. If the correction factors or multivariate correction matrices tested on a daily basis are found to be within the 20% criteria for 5

consecutive days, the required verification frequency of those factors in compliance may be extended to a weekly basis. Also, if the nature of the samples analyzed is such they do not contain concentrations of the interfering elements at ± one reporting limit from zero, daily verification is not required. All inter-element spectral correction factors or multivariate correction matrices must be verified and updated every six months or when instrumentation change, such as in the torch, nebulizer, injector, or plasma conditions occur. Standard solution should be inspected to ensure that there is no contamination that may be perceived as a spectral interference.

17.2.8 Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample or by using a peristaltic pump, by using an internal standard or by using a high solids nebulizer. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, affecting aerosol flow rate and causing instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, using a high solids nebulizer or diluting the sample. Also it has been reported that better control or the argon flow rate, especially to the nebulizer, improves instrument performance: This may be accomplished with the use of mass flow controllers.

17.2.9 Chemical interferences include molecular compound formation, ionization effets and solute vaporization effects. Normally, these effects are not significant with the ICP technique, but if observed, can be minimized by careful selection of operating conditions (incident power, observation position, and so forth), by buffering of the sample, by matrix matching, and by standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

17.2.10 Memory interferences result when analytes in a previous sample contribute to the signals measured in a new sample. Memory effects can result from the sample deposition on the uptake tubing to the nebulizer and from the build up of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples. The possibility of memory interferences should be recognized within an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for a particular element must be estimated prior to analysis. This may be achieved by aspirating a standard containing elements at a concentration ten times the usual amount or at the top of the linear dynamic range. The aspiration time for this sample should be the same as a normal sample analysis period, followed by analysis of the rinse blank at designated intervals.

18.0 Test report

18.1 All information necessary for identification of sample tested (Sample ID, result, method used, date)