



Method number: PV2117

Target concentration: 30 $\mu\text{g}/\text{m}^3$

Procedure: Samples are collected by drawing a known volume of air through a three piece polystyrene cassette containing a glass fiber filter. Samples are extracted with methyl alcohol and analyzed by LC using a UV detector.

Recommended sampling time and sampling rate: 240 min at 1 L/min (240 L) (maximum flow is 2 L/min for 480 L)

Reliable quantitation limit: 1.1 $\mu\text{g}/\text{m}^3$

Status of method: Partially evaluated method. This method has been subjected to established evaluation procedures of the Methods Development Team and is presented for information and trial use.

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1. General Discussion

1.1 Background

1.1.1 History

A request from an OSHA industrial hygienist for a sampling procedure for *l*-thyroxine was received at SLTC. *l*-Thyroxine is a drug used to treat dysfunctions of the thyroid gland. *l*-Thyroxine is prescribed in dosages ranging from 25 to 300 µg, depending on the person's weight and the ability of their thyroid gland to function. A target concentration of 30 µg/m³ was based on the maximum recommended dosage of 300 µg and 10 m³, which is the amount of air the average person breathes in eight hours. Other hormones have been collected on glass fiber filters and analyzed by liquid chromatography (LC) using an ultraviolet detector (see PV 2001 Estradiol, etc.).¹ A C18 column with a mobile phase 80:20:0.2 methyl alcohol:DI water:phosphoric acid at 1mL/min gave a good separation of the *l*-thyroxine peak from the methyl alcohol. The samples were extracted with methyl alcohol, and had extraction efficiencies averaging 100.2% for the concentration range of 0.72 to 14.4 µg/filter. The retention efficiency study showed no *l*-thyroxine on the back up filter for two filters in series that had front filters spiked with 14.4 µg, and had 240-L (at 1 L/min average recovery of 100.4%) or 480-L (at 2 L/min average recovery of 99.5%) humid air drawn through them. The storage study showed no loss for samples stored for up to 14 days under both refrigerated and ambient conditions.

1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.)²

l-Thyroxine is used to treat thyroid gland deficiencies. Overdose symptoms include weight loss, palpitations, nervousness, diarrhea or abdominal cramps, sweating, tachycardia, cardiac arrhythmias, angina pectoris, tremors, headache, insomnia, intolerance to heat, and fever. Prolonged or high overexposure may result in death due to cardiac arrhythmia or failure.

1.1.3 Workplace exposure³

l-Thyroxine and *l*-thyroxine sodium are prescribed for over 9 million patients in the United States. Most tablets consumed in the United States are produced in the United States. Some tablets are exported to other countries. No current data could be found for the number of workers exposed to *l*-thyroxine in the manufacturing process, as the number of companies manufacturing it is ever-changing due to recent FDA regulations. In August 1997, FDA announced that all *l*-thyroxine sodium products were considered new drugs and must undergo the approval process for new drugs even though they were currently being sold. At the time this method was written there were only three forms of *l*-thyroxine sodium that had completed that process, though old stocks of unapproved formulations we still being used.

¹ OSHA Sampling and Analytical Methods. <http://www.osha.gov> (accessed 6/24/2002).

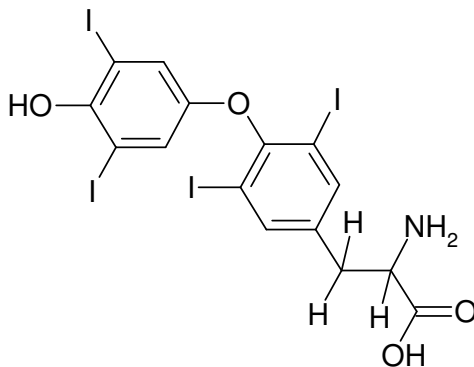
² Arky, R., *Physicians' Desk Reference*, Medical Economics Company: Montvale, NJ, 1997, p 1015.

³ WebMD. <http://www.webmd.com> (accessed 6/25/02).

1.1.4 Physical properties and other descriptive information^{4,5}

CAS number: 51-48-9 IMIS⁶: L200
molecular weight: 776.93 melting point: 223 °C (decomposes)
appearance: off-white to beige solid molecular formula: C₁₅H₁₁I₄NO₄
solubility: water, alcohol, acetone
synonyms: Levothyroxine; / -T4; T4 (hormone); tetraiodothyronine; 3,3',5,5'-tetraiodo-/-thyronine; / -thyroxin; Thyroxinal; Thyroxine; Thyroideum

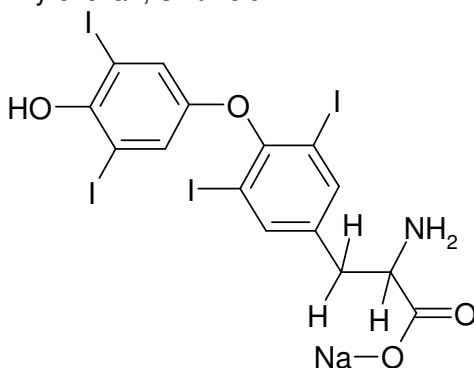
structural formula:



/ -Thyroxine sodium pentahydrate⁷ is the alternate form sold in many pharmaceutical formulations

CAS: 6106-07-6 MW: 888.94
appearance: off-white solid
solubility: water, alcohol, acetone
synonyms: Dathroid; Eltroxin; Euthyrox; Laevoxin; Levaxin; Levoroxine; Levothyroxine sodium; monosodium thyroxine; sodium / -thyroxine; synthroid; Synthroid R; 3,3',5,5'-tetraiodo-Lthyronine, sodium salt; Thyroxevan; Unithroid

structural formula:



⁴ Lewis, R., *Sax's Dangerous Properties of Industrial Materials*, Van Nostrand Reinhold: New York, 2002, p 176.

⁵ Cheminfo <http://cchoh.ca/products/databases/cheminfo.html> (accessed 5/21/2002).

⁶ OSHA Chemical Sampling Information <http://www.osha.gov> (accessed 5/21/2002).

⁷ MSDS Sigma-Aldrich <http://www.sigmaaldrich.com> (accessed 5/21/2002).

This method was evaluated according to the OSHA SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"⁸. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations and acceptance criteria. The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters.

1.2 Detection limit of the overall procedure (DLOP) and reliable quantitation limit (RQL)

The DLOP is measured as mass per sample and expressed as equivalent air concentrations, based on the recommended sampling parameters. Ten samplers were spiked with equal descending increments of analyte, such that the highest sampler loading was 1.06 µg of *l*-thyroxine. This is the amount spiked on a sampler that would produce a peak at least 10 times the response for a sample blank. These spiked samplers were analyzed with the recommended analytical parameters, and the data obtained used to calculate the required parameters (standard error of estimate (SEE) and slope) for the calculation of the DLOP. The slope was 5.18E4 and the SEE was 1169. The RQL is considered the lower limit for precise quantitative measurements. It is determined from the regression line parameters obtained for the calculation of the DLOP, providing 75% to 125% of the analyte is recovered. The DLOP and RQL were 0.0677 µg and 0.266 µg respectively. The recovery at the RQL was 99.5%.

Table 1.2
Detection Limit of the Overall Procedure
for *l*-thyroxine

| mass per sample (µg) | area counts (µV·s) |
|-------------------------|-----------------------|
| 0.00 | 0 |
| 0.106 | 3806 |
| 0.212 | 8629 |
| 0.318 | 13406 |
| 0.424 | 19021 |
| 0.530 | 25092 |
| 0.636 | 30573 |
| 0.742 | 35053 |
| 0.848 | 43231 |
| 0.954 | 48901 |
| 1.060 | 52931 |

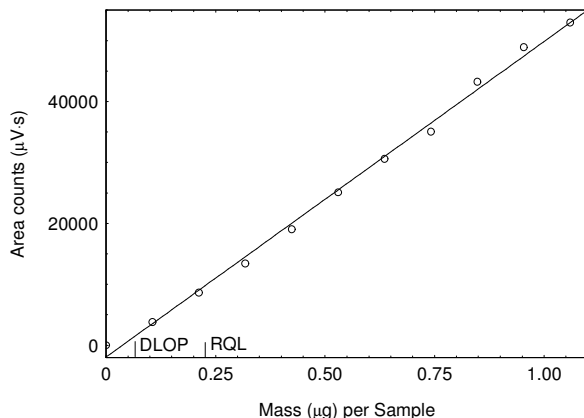


Figure 1.2. Plot of data to determine the DLOP/RQL for *l*-thyroxine at 230 nm. ($y = 5.18E4x - 1927$; SEE = 1169)

⁸ Burrignt, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. C. *Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis*; OSHA Salt Lake Technical Center, U.S. Department of Labor: Salt Lake City, UT, 1999.

Below is the chromatogram of the RQL level.

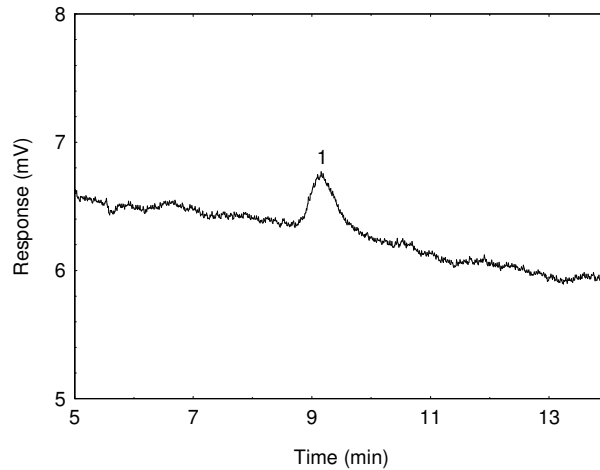


Figure 1.2.2. Chromatogram of the *l*-thyroxine peak in a standard near the RQL at 230 nm. (1 = *l*-thyroxine)

2. Sampling Procedure

All safety practices that apply to the work area being sampled should be followed. The sampling equipment should be attached to the worker in such a manner that it will not interfere with work performance or safety.

2.1 Apparatus

- 2.1.1 Samples are collected using a personal sampling pump calibrated, with the sampling device attached, to within $\pm 5\%$ of the recommended flow rate.
- 2.1.2 Samples are collected with three-piece polystyrene cassettes containing a binderless A/E glass fiber filter. For this evaluation glass fiber filters were purchased from SKC, Inc. (catalog no. 225-7).

2.2 Reagents

None required.

2.3 Technique

- 2.3.1 Immediately before sampling, remove the end plugs from the cassette.
- 2.3.2 Attach the cassette to the sampling pump so that it is in an approximately vertical position with the inlet facing up during sampling. Position the sampling pump, cassette and tubing so it does not impede work performance or safety.
- 2.3.3 Air being sampled should not pass through any hose or tubing before entering the cassette.
- 2.3.4 After sampling for the appropriate time, remove the cassette, and replace the top and end plug. Wrap each sample end-to-end with a Form OSHA-21 seal.
- 2.3.5 Submit at least one blank sample with each set of samples. Handle the blank sample in the same manner as the other samples except draw no air through it.
- 2.3.6 Record sample volumes (in liters of air) for each sample, along with any potential interferences.

2.3.7 Ship any bulk samples separate from the air samples.

2.3.8 Submit the samples to the laboratory for analysis as soon as possible after sampling. If delay is unavoidable, store the samples in a refrigerator.

2.4 Extraction efficiency

The extraction efficiency of *l*-thyroxine was determined by liquid-spiking glass fiber filters with the analyte at 0.1 to 2 times the target concentration. These samples were stored overnight at ambient temperature and then extracted and analyzed. The mean extraction efficiency over the studied range was 100.2%. The wet extraction efficiency was determined at 1 times the target concentration by liquid spiking the analyte onto glass fiber filters which had 240-L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2 °C) drawn through them at 1 L/min immediately before spiking. The mean recovery for the wet samples was 99.8%.

Table 2.4
Extraction Efficiency (%) of *l*-Thyroxine

| level | | sample number | | | | | | mean |
|----------------|---------------|---------------|-------|-------|-------|-------|-------|-------|
| × target concn | µg per sample | 1 | 2 | 3 | 4 | 5 | 6 | |
| 0.1 | 0.72 | 101.5 | 99.4 | 100.9 | 98.7 | 99.1 | 99.6 | 99.9 |
| 0.25 | 1.8 | 99.9 | 100.3 | 101.2 | 100.1 | 99.8 | 98.9 | 100.0 |
| 0.5 | 3.6 | 101.4 | 100.3 | 100.4 | 99.5 | 99.9 | 99.3 | 100.1 |
| 1.0 | 7.2 | 100.9 | 99.2 | 101.5 | 101.0 | 101.4 | 100.8 | 100.8 |
| 1.5 | 10.8 | 101.1 | 100.1 | 100.4 | 99.9 | 100.7 | 100.2 | 100.4 |
| 2.0 | 14.4 | 100.4 | 100.2 | 99.3 | 100.6 | 99.9 | 100.6 | 100.2 |
| 1.0 (wet) | 7.2 | 99.1 | 101.0 | 99.9 | 100.1 | 98.7 | 100.0 | 99.8 |

2.5 Retention efficiency

Six glass fiber filters were spiked with 14.4 µg (60 µg/m³ of *l*-thyroxine and allowed to equilibrate for 4 h. Each spiked filter was placed in a cassette and placed in series with a cassette containing an unspiked glass fiber filter. Each sampling train had 240-L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2 °C) pulled through them at 1 L/min. The samples were extracted and analyzed. The mean recovery was 100.4%. There was no analyte found on the back filters.

Table 2.5.1
Retention Efficiency (%) of *l*-Thyroxine Sampled at 1 L/min

| section | sample number | | | | | | mean |
|----------------|---------------|-------|------|-------|------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| spiked filter | 100.8 | 101.3 | 99.8 | 100.2 | 99.5 | 100.6 | 100.4 |
| back up filter | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 100.8 | 101.3 | 99.8 | 100.2 | 99.5 | 100.6 | 100.4 |

A test to see if a higher sampling rate could be used was conducted by spiking six glass fiber filters with 14.4 µg (60 µg/m³) of *l*-thyroxine and allowing them to equilibrate for 4 h. Each spiked filter was placed in a cassette and placed in series with a cassette containing an unspiked glass fiber filter. Each sampling train had 480-L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2 °C) pulled through them at 2 L/min. The samples were extracted and analyzed. The mean recovery was 99.4%. There was no analyte found on the back filters.

Table 2.5.2
Retention Efficiency (%) of *l*-Thyroxine Sampled at 2 L/min

| section | sample number | | | | | | mean |
|----------------|---------------|------|-------|------|-------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| spiked filter | 100.5 | 97.6 | 100.6 | 99.4 | 100.2 | 98.9 | 99.5 |
| back up filter | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 100.5 | 97.6 | 100.6 | 99.4 | 100.2 | 98.9 | 99.5 |

2.6 Sample storage

Fifteen glass fiber filters were each spiked with 7.2 µg (30 µg/m³) of *l*-thyroxine. They were allowed to equilibrate for 4 h, then 240-L of air, with an absolute humidity of 15.7 milligrams of water per liter of air (about 80% relative humidity at 23 °C), was drawn through them. Three samples were analyzed immediately. The rest of the samples were sealed, and six were stored at room temperature (23 °C), while the other six were stored at refrigerated temperature (4 °C). Three samples stored at room temperature and three samples stored at refrigerated temperature were analyzed after 7 days and the remaining samples after 14 days. The amounts recovered indicate storage was stable at both temperatures for the time period studied.

Table 2.6
Storage Test for *l*-thyroxine

| time (days) | ambient storage | | | refrigerated storage | | |
|----------------|-----------------|-------|-------|----------------------|-------|-------|
| | recovery (%) | | | recovery (%) | | |
| 0 | 101.3 | 100.4 | 101.5 | 101.3 | 100.4 | 101.5 |
| 7 | 100.8 | 100.5 | 101.8 | 101.6 | 101.1 | 100.9 |
| 14 | 101.4 | 101.1 | 100.1 | 100.4 | 101.3 | 101.5 |

2.7 Recommended air volume and sampling rate

Based on the data collected in this evaluation, 240-L air samples should be collected at a sampling rate of 1 L/min for 240 minutes, and for greater sensitivity a higher sampling rate of 2 L/min may be used for 240 minutes for a 480-L air sample.

2.8 Interferences (sampling)

2.8.1 There are no known compounds which will severely interfere with the collection of *l*-thyroxine. Tablets of *l*-thyroxine usually contain a filler. If high loadings of *l*-thyroxine or *l*-thyroxine and filler are expected, sample for less time to avoid clogging the filter with the filler.

2.8.2 Suspected interferences should be reported to the laboratory with submitted samples.

3. Analytical Procedure

Adhere to the rules set down in your Chemical Hygiene Plan. Avoid skin contact and inhalation of all chemicals and review all appropriate MSDSs.

3.1 Apparatus

3.1.1 A liquid chromatograph equipped with a UV detector. For this evaluation, a Waters 600 Controller and pump were used, with a Waters 2487 Dual Wavelength Absorbance Detector, and a Waters 717 Plus Autosampler was used in this evaluation.

3.1.2 An LC column capable of separating *l*-thyroxine from the extraction solvent and any potential interferences. A 4.6 × 250 mm column packed with 5-µm Bakerbond C18 (JT Baker, Phillipsburg, NJ) was used in this evaluation.

- 3.1.3 An electronic integrator or some other suitable means of measuring peak areas. A Waters Millennium³² Data System was used in this evaluation.
 - 3.1.4 Glass vials with poly(tetrafluoroethylene)-lined caps. For this evaluation 4-mL vials were used.
 - 3.1.5 A dispenser capable of delivering 2.0 mL of extracting solvent to prepare standards and samples. If a dispenser is not available, a 2.0-mL volumetric pipet may be used.
 - 3.1.6 Volumetric flasks - 10-mL and other convenient sizes for preparing standards.
- 3.2 Reagents
- 3.2.1 *l*-Thyroxine sodium. Spectrum 99-103% (lot QK0730) was used in this evaluation.
 - 3.2.2 Methyl alcohol, HPLC grade. Fisher 99.9% (lot 023066) was used for this evaluation.
 - 3.2.3 Deionized water (DI water). A Barnstead NANOpure Diamond water deionizer was used in this evaluation.
 - 3.2.4 Mobile phase was 80:20:0.2 methyl alcohol:DI water:phosphoric acid.
- 3.3 Standard preparation
- 3.3.1 Freshly prepare two stock standards. A stock standard may be prepared by weighing out 28.8 mg of *l*-thyroxine in a 10-mL flask, then fill to the mark with methyl alcohol.
 - 3.3.2 Diluted standards are prepared by serial dilution with methyl alcohol. Bracket sample concentrations with working standard concentrations. If sample concentrations are higher than the concentration range of prepared standards, either analyze higher standards, or dilute the sample. The higher standards should be at least as high in concentration as the highest sample. The range of standards used in this study was from 0.1 to 28.8 µg/mL.
- 3.4 Sample preparation
- 3.4.1 Open the cassette and carefully transfer the glass fiber filter to a labeled 4-mL vial. Wipe the inside of the cassette with a glass fiber filter and place into a separate labeled 4-mL vial.
 - 3.4.2 Add 2.0 mL of methyl alcohol to each vial using the same dispenser as used for preparation of standards or use a volumetric pipet.
 - 3.4.3 Immediately seal the vials with poly(tetrafluoroethylene)-lined caps.
 - 3.4.4 Shake the vials on a shaker, or rotate on a rotator, for 30 minutes.

3.5 Analysis

3.5.1 HPLC conditions

column: Bakerbond C18
column 4.6 × 250 mm
injection size: 10 µL
mobile phase: 1 mL/min 80:20:0.2
methyl alcohol:
water:phosphoric acid
detector: UV at 230 and 254 nm
run time: 15 min
retention times: 2.6 min methyl alcohol
9.8 min *l*-thyroxine

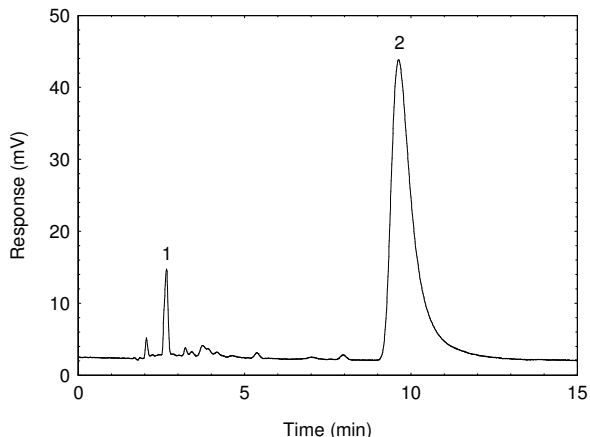


Figure 3.5.1. A chromatogram of 14.4 µg/mL *l*-thyroxine in methyl alcohol at 230 nm. Key: (1) methyl alcohol; (2) *l*-thyroxine.

3.5.2 Peak areas are measured by an integrator or other suitable means.

3.5.3 An external standard (ESTD) calibration method is used. A calibration curve can be constructed by plotting response of standard injections versus micrograms of analyte per sample. Bracket the samples with freshly prepared analytical standards over a range of concentrations.

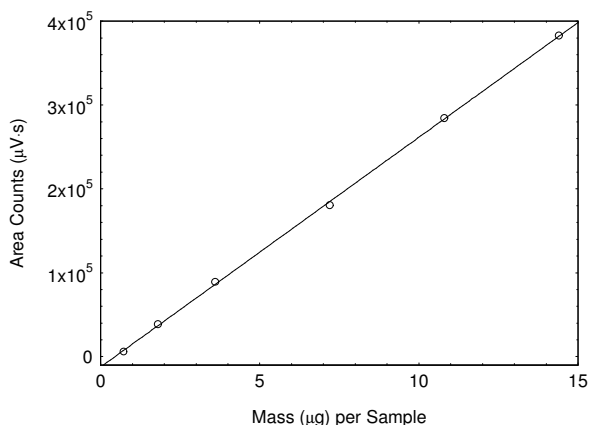


Figure 3.5.2. Calibration curve of *l*-thyroxine at 230 nm. ($Y = 2.74E4x - 1.23E4$).

3.6 Interferences (analytical)

3.6.1 Any compound that produces an LC response and has a similar retention time as the analyte is a potential interference. If any potential interferences were reported, they should be considered before samples are extracted. Generally, chromatographic conditions can be altered to separate an interference from the analyte.

3.6.2 When necessary, the identity or purity of an analyte peak may be confirmed by a photodiode array scan of the peak, by wavelength ratioing, or by LC/mass spec.

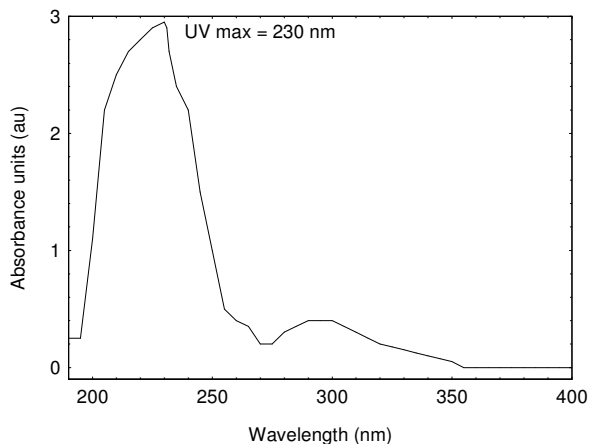


Figure 3.6.2 The UV spectrum of *l*-thyroxine in methyl alcohol.

3.7 Calculations

The amount of analyte per sampler, including the amount found in the cassette wipe, is obtained from the appropriate calibration curve in terms of micrograms per sample, uncorrected for extraction efficiency. This total amount is then corrected by subtracting the total amount (if any) found on the blank. The air concentration is calculated using the following formulas.

$$C_M = \frac{M}{VE_E}$$

where C_M is concentration by weight (mg/m³)
 M is micrograms per sample
 V is liters of air sampled
 E_E is extraction efficiency, in decimal form

4. Recommendations for further study

Collection, reproducibility, and other detection limit studies need to be performed to make this a validated method.