Determination of Mercury in Blood and Urine by Cold Vapor AAS Using the VGA-77

Application Note

Atomic Absorption

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Introduction

This report describes wet chemistry oxidations of blood and urine to produce solutions suitable for analysis with a continuous flow Vapor Generation Accessory, VGA 77. Data are presented to illustrate the performance of the VGA 77 for the determination of mercury in these solutions.

A variety of clinical manifestations of mercury intoxication may be encountered depending mainly upon the mercurial involved. Inhalation of elemental mercury results in pulmonary, central nervous system (CNS) and kidney damage. The CNS damage produces a spectrum of behavioural and neurological deficits. Ingestion of inorganic mercurials may, because of their corrosive nature, produce devastating gastrointestinal damage that is of more immediate clinical importance than the mercury exposure. Once absorbed the inorganic mercurials concentrate in the kidney and may produce a proximal tubular necrosis. Exposure to organomercurials such as methyl mercury produces predominantly CNS effects that are commonly severe and include coma and death. The organomercurials can cross the placenta to the unborn child producing post natal cerebral palsy. Organomercurials are typically encountered in the food chain as a result of methylation of inorganic mercury salts by bacteria. There have been reports of various clinical presentations where pharmaceutical organomercurials have been applied to surgical wounds and membranes as desiccants and antiseptics [1].

Requests for mercury determination are triggered in three main ways. Workers in known mercury containing industrial environments should be regularly monitored for exposure. Urine is the most suitable matrix. Known exposure to mercurials whether by accident or design should be assessed by determination in urine, blood and possibly hair.

Subjects presenting at medical examination with CNS or peripheral dysfunction whether or not accompanied with gastrointestinal or renal complications are candidates for mercury exposure assessment. These cases are unlikely to be acute in nature; urine and possibly hair are the matrices of choice.

Agilent Technologies

Experimental

Instrumentation

The VGA 77 [2] (Figure 1) is a continuous flow device that uses a peristaltic pump to mix reagent and sample streams with a supply of inert gas. The inert gas and any volatiles are stripped from the liquid in a gas-liquid separator. The separated gas flows into a flow-through cell aligned on the light path of an atomic absorption spectrometer. The accessory may be used to generate volatile mercury or hydrides from solution. In this laboratory the VGA 77 is operated with an atomic absorption spectrometer equipped with deuterium background correction.

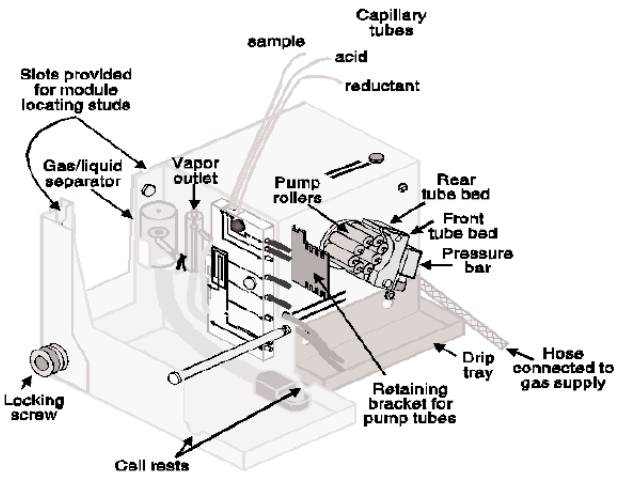

Figure 1. The Vapor Generation Accessory VGA 77

The spectrometer is operated at the 253.7 nm line with a hollow cathode current of 5 mA and a slit width of 1.0 nm. Three readings of the steady state signal are taken over a period of 9 seconds, the steady state having been achieved one to two minutes after sample uptake has commenced. These triplicate readings should have a relative standard deviation of less than 5% where the observed absorbances are greater than 0.010.

Reagents and Solutions

Sample volumes can be restricted in some clinical scenarios so the methods presented here have been scaled to provide just sufficient test solution for the VGA 77 to generate an acceptable analytical signal of sufficient duration to be measurable with the atomic absorption spectrometer.

Urine

The technique reported here is a derivation of a US-EPA method for waters and wastes [3], the major alteration being sample volume reduction to the levels which may be required when servicing a paediatric population. 2.00 mL of urine is mixed with 0.100 mL of 35% w/w nitric acid, 0.200 mL of 50% w/w sulfuric acid and 0.500 mL of 5% w/v potassium permanganate. The reagent/sample solution is allowed to stand at room temperature for 15 minutes. If the solution's color has changed from purple to brown then a further 0.500 mL of the permanganate solution is added, mixed and allowed to stand for a further 15 minutes. This process of adding successive aliquots of permanganate solution and allowing the reaction to proceed is maintained until the purple color is sustained. With increasing masses of dissolved organic materials increasing volumes of permanganate solution will be required. After the permanganate reaction is complete add and mix 0.400 mL of 2.5% (w/v) potassium persulfate. Incubate at 85–95 °C for at least 90 minutes and then let cool. Add and mix 0.5 mL each of butan-1-ol (used to control foaming) and 5% (w/v) hydroxylamine hydrochloride in 3% (w/v) sodium chloride. Make the total volume up to 10.0 mL with reagent water and mix well prior to determination.

Blood

This sample matrix cannot be successfully digested by the procedure described for urine. The mineral acid digestion described is a variation of a published technique [4]. 1.00 mL of anticoagulated whole blood is mixed with approximately 3 mL of a 5:2:1 mixture of 70% (w/w) nitric acid, 70% (w/w) perchloric acid and 98% w/w sulfuric acid in a 10 mL nominal-volume graduated polyethylene test tube. The digestion mixture is warmed to 40 °C for 60 minutes and the heated at 90 °C for at least 60 minutes with frequent mixing until the brown fumes of oxides of nitrogen dissipate and the remaining liquid is golden yellow in color. It is important to control the liquid's temperature so that boiling any stronger than could be described as effervescence is precluded. Make up to 5.0 mL with reagent water. Mix the solution thoroughly prior to determination.

Reagents for the VGA-77

Ionic mercury in the test solutions is reduced to metallic mercury by sodium borohydride in a hydrochloric acid matrix. The reductant solution used is 0.6% sodium borohydride in 0.6% sodium hydroxide. The acid solution is 18% w/w hydrochloric acid. Two drops of secondary octyl alcohol are pipetted into the gas-liquid separator prior to commencing the analysis to control foaming.

Calibration

Urine

Urine determinations are calibrated by preparing a series of additions (up to 400 nmol/L) to a representative urine that contains minimal endogenous mercury. This calibration set should be freshly prepared for each analytical run and be digested in parallel with the test samples and controls.

Blood

Blood determinations are calibrated by preparing a series of mercury solutions (up to the equivalent of 500 nmol mercury per litre of blood) in 0.4% (w/w) nitric acid and 1% (w/w) sulfuric acid and perchloric acid. This calibration set should also be freshly prepared for each analytical run.

Quality Control and Assurance

Blanks, spike recoveries and certified or survey validated matrix matched controls should be included in each analysis run. Laboratories undertaking these determinations should also participate in at least one external quality assurance scheme. The measurement of an analysis blank is precluded in the blood determination because of the relatively high acid content of such a sample relative to the calibrators and digested samples. It is strongly recommended that a known mercury-free blood be determined at regular intervals to maintain control over reagent contamination levels.

Results and Discussion

Recovery of Added Mercury

Urine

Urine determinations are calibrated using the (non-rigorous) method of analyte additions. Because of the known matrix variability, test samples are determined in duplicate with one of the test pair having an addition of 50 nmol mercury per litre. The average recovery of this addition over the past 6 months has been 99% with a standard deviation of 10%.

Blood

Blood determinations are calibrated by aqueous acid balanced standards as sample volume limitations preclude the method of additions. The assessment of spike recovery in this case is therefore more important. Test samples are again determined in duplicate with one of the pair being spiked. The average recovery of a 50 nmol/L addition of mercury over the past 6 months has been 103% with a standard deviation of 15%.

Recovery of Added Organomercurials

Methylmercuric chloride and merbromin (dibromohydroxymercurifluorescein) were used as indicators of the method's recovery of organomercurials.

Methylmercury is a metabolite of inorganic mercury and is the most commonly found organomercurial in marine biota. Merbromin is used as a topical disinfectant and desiccant in clinical medicine. In these applications it is in direct contact with open lesions and may potentially be transferred to the systemic circulation.

Reasonable recovery of these and like compounds is therefore necessary where methods are applied to biological fluids. The observed recoveries of these compounds over two full procedures is shown in Table 1.

Table 1. Recovery of Organomercurials

Urine		Blood	
merbromin	methyl-mercury	merbromin	methyl-mercury
75%	66%	77%	71%

Controls – Urine Determination

The determination is controlled by inclusion of two commercial control samples with each analytical run. Performance data for the past 12 months is in Table 2.

Table 2. Urine Mercury Performance as Assessed by Commercial Controls

Material	Target (acceptable range)	Achieved (mean)	Achieved (standard deviation)
Biorad Lyphochek Level 1 Lot No. 62021	0.06 µmol/L (0.05 - 0.08)	0.063 µmol/L	0.009 µmol/L (n=60)
Biorad Lyphochek Level 2 Lot No. 62022	0.76 µmol/L (0.61 - 0.91)	0.700 µmol/L	0.085 µmol/L (n=62)

The laboratory participates in a quality assurance program distributed by Quality Control Technologies [5]. One sample is received for mercury analysis each month, results for the past 34 months are shown in Figure 2.

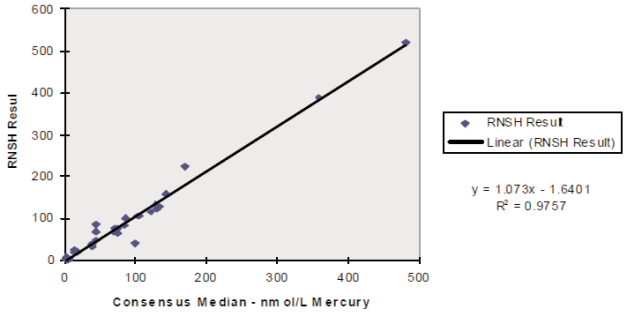

Figure 2. Urine mercury performance as assessed by external quality assurance (September 1994 to July 1997, 32 points) controls – blood determination.

This determination is controlled by the inclusion of (retained QAP) survey validated samples in each analytical procedure. Performance data for the last 34 months are shown in Figure 3.

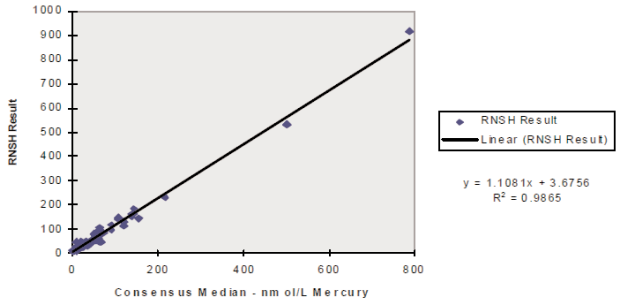

Figure 3. Blood mercury performance as assessed by retained survey validated samples (September 1994 to July 1997, 67 points).

The laboratory participates in the blood mercury quality assurance program distributed by Quality Control Technologies [5]. Three samples are received each month. Results for the past 34 months are shown in Figure 4.

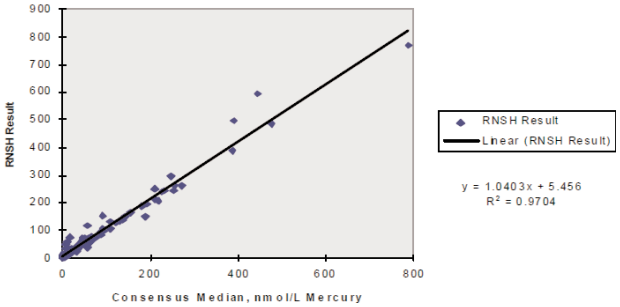

Figure 4. Blood mercury performance as assessed by external quality assurance (September 1994 to July 1997, 102 points).

Conclusion

The methods presented here for the determination of mercury in urine and blood, and the VGA-77, have proved to be reliable and robust.

Precision and accuracy, as tested by external proficiency programs, have been found to be completely adequate for screening for mercury exposure in a clinical environment where sample mercury concentration may be expected to be low and sample volumes restricted.

The observed recovery of organomercurials is less than ideal and does not support previous publications that describe similar techniques. The reason for these findings is unclear. The observed recoveries are sufficient for the methods to be applied in accident and emergency medicine.

Routine operation of the VGA-77 in association with an atomic absorption spectrometer is simple and inexpensive. No breakdowns have occurred in 36 months of operation, pump tube renewal being the sole maintenance requisite if the system is well flushed with water after each procedure.

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