Validation of an Automated System for Methyl Mercury Analysis using Cold Vapor Atomic Fluorescence Spectroscopy with Gas Chromatography Separation

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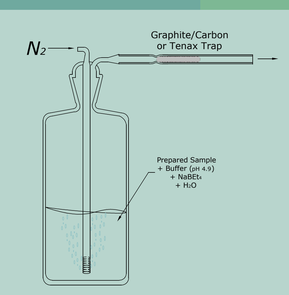
A system was devised to allow automated analysis of prepared samples for the determination of methyl mercury. This system is based on an established EPA method (1630).

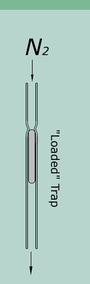
MANUAL PROCESS of ANALYSIS (per EPA Draft Method 1630)

PURGE &TRAP



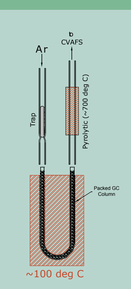
Buffer is added, then ethylating reagent, and given time to react (~17 min). Nitrogen gas is purged through the solution and the volatile components collected on the trap (~17 min).

TRAP DRYING



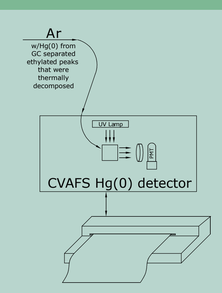
Using nitrogen gas, the "loaded" trap is then dried before analysis (~7 min).

DESORBTION, GC SEPARATION & THERMAL DECOMPOSITION



Ethylated compounds are released from the trap by rapid heating then separated from each other in the GC column. All mercury compounds passing through the pyrolytic column are decomposed liberating elemental Hg for detection.

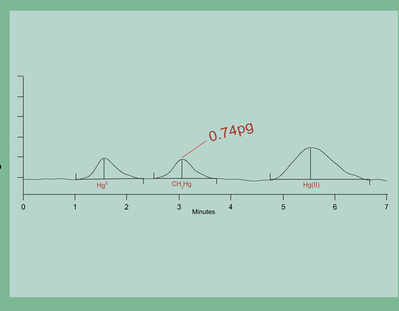
DETECTION BY CVAFS & PHYSICAL MEASURMENTS OF CHART RECORD PEAKS



Hg(0) is continuously measured by the CVAFS, giving a relative measure at any given moment. Peaks resulting from GC separated compounds are manually measured in height to provide a result.

TYPICAL OUTPUT

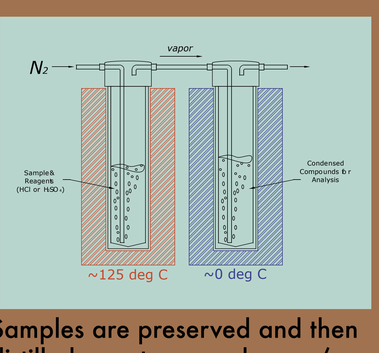
RESULTS



Using the measurement from the peak of interest, the quantity of mercury for a given run is calculated based on standard and blank run results. Results for samples, standards, blanks, and QC runs are processed for the batch manually by the technician.

SAMPLE PREPERATION

Distilation



Samples are preserved and then distilled over to a condenser / receiver. The condensed liquid is subsequently used for analysis.

Same preperation regardless of method of analysis

Manual Process: Slow, tedious, and prone to errors at each step.
vs.
Automated Process: Higher throughput, easier reproducibility, less manpower wasted on repetitious tasks.

AUTOMATED PROCESS of ANALYSIS

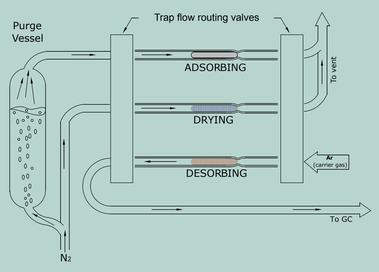
VIAL LOADING WITH PRE-ANALYSIS ETHYLATION

Prepared samples are buffered in vials and then ethylating reagent in added. The vials are topped off and capped with a septa lid. Preventing any air bubbles is a requirement.

AUTOMATED SAMPLER TRANSFER OF LIQUID

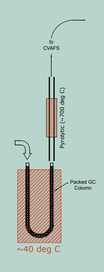
Under computer control, the autosampler will extract the liquid for analysis during the batch run. The liquid is transferred to the purge vessel and then purged (~5 minutes).

SIMULTANEOUS MULTIPLE TRAP PROCESSING



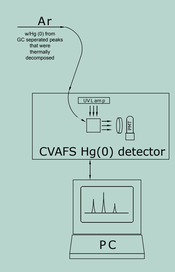
Nitrogen purge gas strips the volatile ethylated species so that they can be adsorbed onto a trap. Drying of a second trap and desorption of a third are all carried out simultaneously. The desorption trap is heated rapidly to release the ethylated compounds. (Cycle time ~6 min.)

GC SEPARATION & THERMAL DECOMPOSITION



Ethylated compounds from the desorption trap are separated from each other in the GC column. All mercury compounds passing through the pyrolytic column are decomposed liberating elemental Hg for detection.

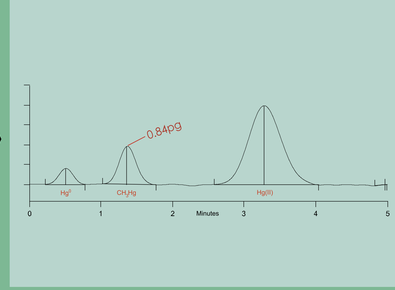
DETECTION BY CVAFS & PEAK EVALUATION BY COMPUTER



Hg(0) is continuously measured by the CVAFS, giving a relative measure at any given moment. Peaks resulting from GC separated compounds are automatically measured in height or area by software to provide results.

TYPICAL OUTPUT

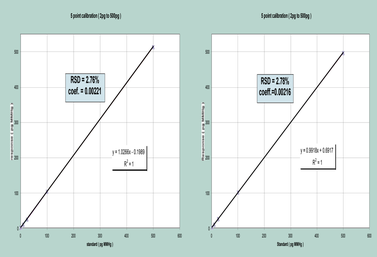
RESULTS



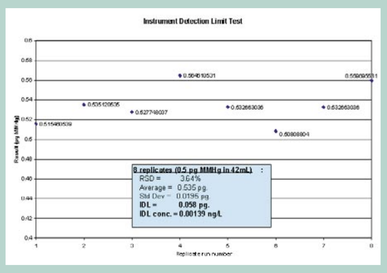
Using the measurement from the peak of interest, the quantity of mercury for a given run is calculated based on standard and blank run results. Results for samples, standards, blanks, and QC runs are processed for the batch under software control.

Addition of ethylating reagent into the vial, ahead of sealing and subsequent purging, was found to be stable and effective as long as no air was trapped within the vial. In the presence of air this highly reactive reagent was found to be adversely affected and lower recoveries resulted. Once careful attention was paid to issues such as this, the system was found to work reliably and provided results as good or better than from manual analysis. Considerably less human labor is required as most repetitious tasks are no longer the responsibility of the technician.

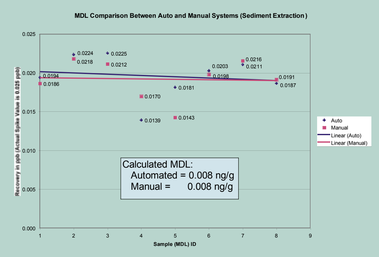
We investigated a number of variables in order to try and discover influential parameters unique to the capped ethylation and postponed purging technique that we employed. A subset of results from preliminary work to investigate these effects and to determine general performance of our automated system are shown below:



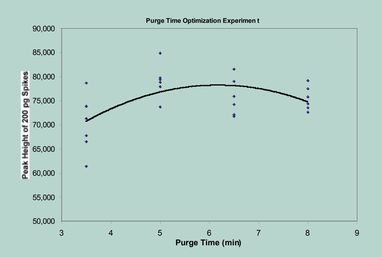
Two five-point calibrations (2pg, 10pg, 25pg, 100pg, 500pg) were conducted within a single batch on the automated system. Results from both batches matched each other quite closely with both giving excellent RSD and linearity. This is comparable to the best results found when calibrations are performed manually.



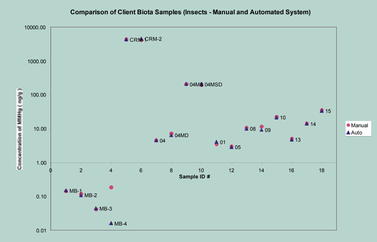
An instrument detection limit study was performed. Eight replicates at 0.5 pg were used and four mid level spikes at 5pg. The mid level spikes came out between 97% to 115% with an RSD of 7.8%. The IDL was calculated by multiplying the standard deviation of the eight 0.5 pg replicates by the student t value of 2.998. The resulting IDL was 0.058 pg. MMHg (1.39 pg/L for 42mL analyzed).



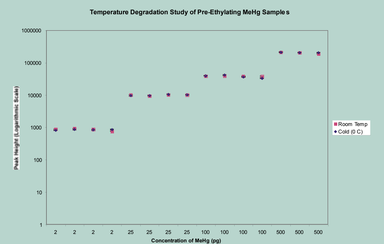
Client sediment samples were prepared using EPA Method 1630 (BRL equiv. SOP BR-0011). Aliquots were taken and analyzed using EPA Method 1630 with manual and automated system analysis procedures. Both gave a method detection limit of 0.008 ng/g. This corresponds to an RSD of 10% for a total of 8 samples. This MDL was slightly higher than our current MDL at BRL, 0.006 ng/g. Given that the sediment extraction is quite involved and lengthy, this preliminary result is satisfactory.



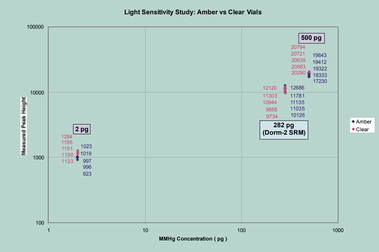
Six replicate runs were conducted for each of four differing purge times (3.5, 5, 6.5, and 8 min. ; drying time = 5 min.). An increase in measured MMHg quantity occurred with longer total purge time, as anticipated since this allows more complete stripping from the solution. With increasing total gas volume, there were losses from ethylated MMHg migration out of the trap.



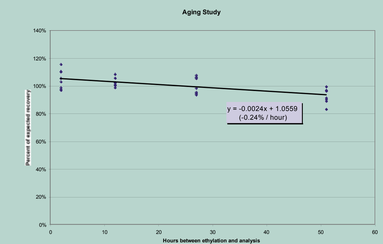
Each sample of well homogenized insect was digested/extracted using KOH/methanol, heated at 65 deg. C, and further diluted with methanol. Certified reference material (Dorm-2) was prepared in the same way. Aliquots were analyzed using both manual techniques and with the automated system. Results were in good agreement except for method blank 4 on the automated system that may not have been ethylated.



In order to examine the possible degrading effects of temperature on pre-ethylated samples, an experiment was conducted where pre-ethylated standards (ranging from 2pg-500 pg) were refrigerated for 48 hours. Control samples were also pre-ethylated in the same manner but kept in a drawer at room temperature. Both sets were analyzed in the same batch. Results indicated no significant effect on pre-ethylated samples between room temperature and 0 deg C.



Two sets of identical standard samples were prepared in clear and amber vials. Each was spiked with an aliquot of either a standard or CRM (Dorm-2). Vials were stored for 48 hours in a bright North facing window during summer such that they would be exposed to bright diffuse natural light. The results did not indicate that exposure to light causes degradation of the MMHg but further investigation with various real world samples will be more conclusive.



Eight replicate samples of Dorm-2 (Dogfish muscle tissue digested extraction) were ethylated in amber vials at various times ahead of analysis (approx. 2, 12, 27, and 51 hrs). Storage was at room temperature and no precautions made to avoid exposure to ambient light. Using averaged value results, there was degradation in response on the order of 0.24% per hour of aging time.

Summary: We found that by optimizing the system components, providing computer controlled automation, and modifying the standard methodology slightly, it is possible to get excellent results. Our findings up to this point have yet to show any significant limitation that might prevent analysis of a particular type sample matrix that is currently performed successfully with manual procedures.

Advantages over the existing manual method are easier reproducibility of results with greater throughput and reduced requirement of analytical expertise. Maintenance of the system has been found to be minimal and sources of potential errors are well understood. Refinements of every part within the analytical process and elimination of steps that introduce human errors has lead to results that are found to be as good or better than those resulting from analysis using manual procedures.

48 run batch comparison (person hours)

Manual: 8+ hrs. continuous attended operation
Automated: <2 hrs. pre-analysis, unattended afterward