June 13, 2007

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Beta Site Evaluation of the EPA Carbamate in Drinking Water Method 531.1 with Pickering Laboratories Pinnacle PCX Integrated LC/ Post-Column Derivatization Instrument

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Introduction

The Nebraska DHHS Public Health Environmental Laboratory conducts environmental testing for the state of Nebraska. It employs mandated U.S. Environmental Protection Agency (EPA) methods for the determination of regulated substances such as carbamates in drinking water. The drinking water program division has to ensure that drinking water is safe and meets detection limit criteria. The Safe Drinking Water Act Testing (SWDA) is provided to municipalities in accordance with federal regulations. The laboratory employs certified EPA Methods to determine if contamination levels exceed regulated maximum contamination levels (MCs) L. These methods are designed to detect the stated drinking water contaminants within the specified detection limits published in the Federal Register. All procedures are conducted under compliance requirements with audit tracking. In order to ensure that the laboratory is meeting the protocol requirements of both Method and Laboratory practice, the laboratory annually undergoes internal audits conducted by qualified Nebraska DHHS Public Health Environmental Laboratory staff as well as external EPA audits. Typical analyses conducted by HHS-R&L include diquat and paraquat (USEPA Method 549.2), Roundup, a glyphosate in drinking water, (EPA Method 547) and carbamate (EPA Method 531.1.) Measurement of N-methylcarbamoyloximes and N-methylcarbamates in drinking water is performed by direct injection HPLC with post-column reaction derivatization. The water sample is filtered and 30 µL is injected into a reverse phase HPLC column. Separation of the analytes is achieved using a methanol/water gradient protocol. The eluted analytes are hydrolysed using Pickering hydrolysis reagent at 100° C. The methylamine formed during the hydrolysis is reacted with o-phthaladehyde and the nucleophile, 2-mercaptoethanol (or nucleophilic Pickering Thiofluor) to form a highly fluorescent isoindole derivative. The optimized Pickering carbamate analytical system allows one to detect a spectrum of carbamates at ppb (ug/L) levels in drinking water. An Agilent 1100 HPLC interfaced with a Pickering Pinnacle PCX instrumentation system was used for the beta-site study.

Sample Collection and Analysis Protocol Overview

Samples collected for the determination of carbamate target analytes consist of 2-40 mL glass vials filled with sample plus a field reagent blank (FRB). Laboratory milli-Q reagent water is used for the FRB and is treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes are present in the field environment. All samples are preserved by adding 1.2 mL Pickering Chlorac buffer per 40 mL sample to attain an optimum pH of 3.0. The Pickering Chlorac preservative is a specifically purified grade of chloroacetic acid that eliminates baseline noise and interfering peaks. This allows one to achieve low detection limits and sensitivity that required by EPA 531.1. If a sample is taken from a chlorinated source, the samples vials and field reagent blank must contain 5 mg of sodium thiosulfate prior to collecting the sample.

A routine analysis sequence includes 20 environmental samples plus the necessary standards and quality control samples (QCS). To establish initial calibration verification, the highest calibration standard (5 µg/L) is injected three times. Usually the highest calibration standard is within 90 % of the expected value. If a period of 4 weeks or greater has elapsed since the last 6 standard level calibration (0.5, 1.0, 2.0, 3.0, 4.0, and 5 µg/L) was done, then a full range 6 level calibration curve must be performed. The % RSD (relative standard deviation) of the response curve for each calibrated carbamate analyte is typically 5% or less for Agilent LC1100 / Pickering PCX system. An average response factor is used for quantitation of carbamates in unknown and control samples.

The following quality control samples are analyzed: LPC, LFB, LFM, LRB, FRB, QCS.

Laboratory Performance Check Solution (LPC): A solution of method analytes, surrogate compounds, and internal standards used to evaluate the Agilent LC1100 (Agilent Technologies, Palo Alto, CA)/ Pinnacle PCX instrument system with respect to a defined set of method criteria. A commercial LPC containing aldicarb sufoxide at 100 µg/L 3-Hydroxycarbofuran at 2 µg/L, methiocarb at 20 µg/L, and BDMC (4-Bromo-3,5-Dimethylphenyl N-methylcarbamate) at 10 µg/L is used to evaluate the instrument system. Sensitivity, chromatographic, and column performance criteria specified in EPA 531.1 must be met. Sensitivity (S/N >3) for 3-Hydroxycarbofuran at 2 µg/L, Peak Gaussian Factor (0.90<PGF<1.10) for aldicarb sulfoxide, resolution of methiocarb-BDMC greater than 1.0 (1).

Laboratory Fortified Blank (LFB): An aliquot of blank sample matrix (Fisher Scientific HPLC grade water) fortified at 2 µg/L for method analytes (aldicarb, aldicarb sulfone, aldicarb sulfoxide, propoxur, arbyl, carbofuran, 3-hydroxycarbofuran, methiocarb, methomyl, oxyamyl, bdmc surrogate) is analyzed exactly like a sample and is used to determine whether the laboratory is capable of making accurate and precise measurements at the required method detection limit (MDL). A low level laboratory fortified solution LLLFB (0.2 or 0.4 µg/L) is more representative to check accuracy and precision of analytical system at or near the MDL. Usually the LLLFB is at or less than the lowest calibration standard (0.5 µg/L). Statistics on routine measurements of the LLLFB allow one to validate the MDL with each analysis sequence (analytical bias or inaccuracy and precision near the MDL).

Laboratory Fortified Matrix Sample (LFM): An aliquot of environmental sample fortified at the same level as the LFB with known quantities of method analytes. The LFM is analyzed in the same manner as a regular sample and is used to evaluate whether sample matrix contributes to bias to the analytical result. If a LFM duplicate is analyzed, important precision in recovery of method analytes can be obtained for real world samples in the presence of matrix interferences.

Laboratory Reagent Blank (LRB): An aliquot of laboratory reagent water that is treated exactly and a sample, including glassware, solvents, equipment, surrogates, etc. The LRB determines if method analytes or other interferences are present in the laboratory environment, reagents, etc.

Quality Control Sample (QCS): A control sample containing method analytes obtained from a source external to the laboratory and used to check laboratory performance. The QCS should be a test material from a different source than the calibration standards. Historical performance evaluation test samples can be used (PT samples). Once initial calibration verification has been performed, an analysis sequence can be established.

Since the analysis sequence will run for more than 24 hours, a periodic check calibration standard (2 µg/L) is run after every 10 samples. A sequence of 20 samples will therefore contain 3- 2 µg/L level calibration, which can be used to calculate a 3 standard deviation retention time window and to calculate the % difference in analyte response factors. The % difference in analyte response factor needs to be less than 10%. The % difference calculation uses the absolute difference in response factors for the periodic check standard and the average analyte response factor from the 6 level standard calibration curve divided by the average response factor times 100.

Confirmation for the presence of any carbamate found using the water/methanol gradient method is done by using a water/acetonitrile gradient system. One can use a different Pickering reverse phase column to accomplish the confirmation: 5µm C-8 4.0 x 250 mm or C-18 4.6 x 250 mm column. If the C-18 is the primary column for the water/methanol gradient system, then a C-8 column is used for the water/ acetonitrile confirmation procedure. The same calibration standards and QCS samples need to be processed for the confirmation sequence. The suspect carbamate anayte must be within the 3 standard deviation retention time window for both routine and confirmation methods. The Pickering carbamate columns are optimized for USEPA 531.1. If one replaces an existing old column with a new column, the same gradient program specified by Pickering can be used without any changes. Chromatographic performance parameters such as peak shape, peak width, column efficiency, analyte retention time, selectivity, etc. are reproducible. Although most reverse-phase columns survive about 500 injections, the Pickering columns have been used for almost 1000 injections for a 30µL injection volume. Good low level analyte detection, reproducibility, and sensitivity are still apparent after 500 injections. The Nebraska DHHS Public Health Environmental Laboratory analyzes about 800 carbamate environmental ground water samples per year during an active monitoring period.

Carbamate Unknowns Determined

At present, only a few carbamate analytes are regulated (Table 1). We have calibrations for all of these. There has been some talk that the number of regulated carbamate analytes will be expanded following a European Union (EU) lead. In order for such a change to occur, it would first be published as a notice in the Federal Register. There would also be a new draft of the procedure that included the additional analytes. The laboratory would then have to certify each analyte tested. Currently, we are not directed to develop new methods or applications.

Analysis/Conditions/Reagents

While the laboratory could make up its own reagents, the carbamate reagents used for Pinnacle PCX analyses conducted were purchased from the instrument manufacturer. One of the attractive features about the Pickering reagents is the fact they are packaged with lot numbers and expiration dates. This makes them especially useful for audits as they are certified for the application, e.g., carbamate. In principle, one can use any column that meets the test requirements. Again, the Pickering columns provide an advantage in that they are optimized for the specific analytical application and this saves time as there is no need to adjust the gradient program. The question of optimization is important. While the chemistry may not be altered, it is possible to optimize the procedure, e.g., fine tune the flow rate, etc. One also needs to ensure that the preservative is effective and that there is no hydrolysis or other chemical transformation. For example, aldlicarb (a regulated carbamate) might oxidize to the corresponding oxide (a different regulated carbamate) and the result would be an inversion of the concentration relationship between the respective peaks. (i.e., normally the oxide peak would be smaller). Pickering addresses this contingency in some of their research communications.

Background

The Pinnacle PCX is the third generation of Pickering PCD instruments that have been used in this laboratory. Initially, we began working with the PCX 5100 that eventually was replaced by the PCX 5200, its successor, an instrument that has provided excellent performance for a 5-year period. We believe the consistently good performance of the PCX 5200 was the result of good instrument design coupled with attention to performing regular maintenance.

Overview of the Pinnacle PCX Technology

There are some obvious hardware differences between the new Pinnacle PCX and its predecessor, the PCX 5200. For one, the older system is equipped with a reciprocating pump that uses check valves while the new system uses a syringe pump. It is possible that some of the occasional leaks experienced with the PCX 5200 might have been avoided with Pinnacle PCX pump hardware; however this can only be ascertained over time. In any case, the new system design does provide easier access to hardware components. As a result, changing the reaction coil is facilitated, which is helpful when changing methods. This was not the case with the older system, which required removal of the front panels in order to gain access to these components.

System programming improvements in the Pinnacle PCX are also evident, for example, in switching from a carbamate to a glyphosate analysis. The Pinnacle PCX syringe pump provides the capability for a piston actuated wash/reagent flush. This is conveniently performed and the method can be set up in software to accomplish this. The Pinnacle PCX is also equipped with a column convection oven which makes it easier to rapidly cool the column. Thus, in switching from carbamate to Glyphosate methods, the column temperature can be rapidly equilibrated, which translates to an improvement in throughput. Moreover, cooling and system flush are carried out simultaneously, further decreasing the switching interval between carrying out the two types of PCD analyses on this system. Currently, when running a sequence, the shut down procedure equilibrates at the temperature specified. We use a generic piston seal wash/reagent flush for both carbamate and glyphosate methods - 15% methanol/ water. Another benefit attributed to the new syringe pump is conservation of reagent, i.e., when the column is equilibrating it is not pumping reagent. This is actually a significant issue as in addition to the cost saving, the disposal of toxic reagent materials can be problematic and the opportunity to minimize the size of toxic waste is a welcome result.

While the system automation provides additional flexibility and reduces the changeover interval between methods, it cannot speed up a run per se. This can only be achieved by moving to a shorter column and at present the EPA default protocols do not specify 50 mm microbore columns. Currently, we use columns with dimensions of 150-250 mm x 4.6 mm. The constraints imposed with environmental EPA methods compel the use of "classical" technology, even with "smarter" systems. Eventually, one can anticipate moving to performance-based methods. Even then, it would be necessary to ensure that requirements such as detection limits are not compromised

System Performance

Certain basic approaches are systematically applied in evaluating analytical equipment. Accordingly, the system was challenged to evaluate the linearity of the calibration curve, to ensure a sufficient performance quality and to verify that it meets specifications. In that regard, the Pinnacle PCX meets the EPA detection limits for drinking water (Table 2). Beyond this benchmark, the capability to exceed these specifications would depend on the total system. With an Agilent 1100 series LC and Agilent 1100 fluorescence detector, the detection limits are found to be very good just considering the LC system alone. When evaluating the ultimate sensitivity of a detector, a major concern is baseline noise. Moreover, detection limits can change (for better or for worse) as a column ages, system tuning changes, etc. One must be aware that detection limits have statistics as well and that these can vary.

From our observations it appears that the Pinnacle PCX detection limits are equal to or superior to its predecessor*. This is a rough observation since the EPA method does not evaluate sensitivities below that stipulated by the protocol. In the current case, it appears that baseline noise is a bit lower. It is desirable that the detection limits be considerably lower than the natural contamination level; say 5- to 10-fold lower. These detection limits are not static numbers and can vary + 20 %. Beyond this rough approximation, more time would be required to completely evaluate relative system performance. However, it does appear that carbamate sensitivity is improved. Overall, however, this factor does not carry much weight with regard to the results we are seeking. In that respect what can be verified is that the new system meets those requirements. It must be remembered that although EPA protocols set mandatory detection limits, they are not designed to determine an instrument's limit of detection (LOD).

**These values were obtained using injection volumes of 30 uL, which is impressive since common injection volumes for carbamate in drinking water analysis is typically 400 uL. Using smaller injection volumes has the added benefit of extending column life and improving resolution, especially in the beginning of the chromatogram.*

Carbamate concentrations can go down to 0.2 ug/L for the carbamate procedure. Each time we run a procedure we test below the lowest standards - a kind of quality control of the detection limit. It is obvious that at this level the sensitivity has to be extraordinary for carbamates. While the lowest concentration calibration standard is 0.5 ug/L, we can get good quality control in terms of precision, that is, accurate results at 0.2 ug/L and 0.4 ug/L. Good precision at these very low concentration levels is one of the things that is very impressive about Pickering technology. Typical QC charts (fig.1) indicate some scatter in these measurements, but the precision is quite respectable considering the concentration. Obviously the precision will not be as good at a fortification level of 0.2 ug/L and 0.4 ug/L as at 2 ug/L (fig.2) or 5 ug/L. Below 0.2 ug/L, one might say that if the scatter is reflective of variation in actual sample concentration and not in instrument sensitivity, then sensitivity actually approaches 0.1 ug/L (fig.1). However, this is an observational extrapolation and not a verified determination. It must be remembered that at the limit of detection, small, day to day variations in instrument tuning, baseline noise, etc., are consequential. In any case, these results indicate that the instrument performance as tested is very accurate and reproducible

Conclusion

As of this writing, the Pinnacle PCX has been in place for about 15 months at our laboratory and we have analyzed approximately 1,000 carbamate environmental samples with it. The new system definitely provides an improvement in convenience: Switching columns, for example, ease of use, time saved and flexibility all appear to be enhanced. Robustness would need a longer evaluation term but so far, performance is flawless. Pinnacle PCX post column technology is simple enough to be employed by an operator with a limited amount of experience, particularly if one uses it with Pickering standardized reagents, because the system's already optimized for the prepackaged chemistry. There's also the matter of maintenance convenience. All that is required to ensure that problems are quickly addressed with minimal downtime is to perform the necessary reagent flushes and follow the protocol strictly. From the perspective of sensitivity, the new instrument is capable of carbamate detection limits equal to or better than its predecessor. As constructed, the Pinnacle PCX enables carrying out the necessary workflow in a very economical and productive manner for the carbamate and other analyses required by the Safe (Drinking) Water Act—EPA protocol, which we perform.

About the Author:

Dr. James Balk, (M.S., Ph.D, Biochemistry, University of Nebraska , MT (ASCP) Medical Technology , Bryan Memorial Hospital , Department of Pathology) is a chemist for the Program Division responsible for EPA mandated analyses for drinking water at the Nebraska DHHS Public Health Environmental Laboratory. Dr Balk's experience includes 25 years at Nebraska DHHS Public Health Environmental Laboratory which includes 9 years in HPLC and 16 years in radiochemistry. Additionally, he has a span of 16 years covering academic and research experience in clinical chemistry, pharmacology, and medical technology.

References

1. Standard Operating Procedure, Measurement of N-Methyl Carbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection, EPA Method 531.1, Revision 3.1 (1995), HPLC with Post Column Derivatization, HHS R & L Laboratory Sop #3310.B, August 2001, http://www.ultrasci.com/docs/analyticalmethod/method14.pdf

2. J.W. Munch (1995), METHOD 531.1, Measurement Of N-Methylcarbamoyloximes and N-Methylcarbamates In Water by Direct Aqueous Injection HPLC with Post Column Derivatization, Revision 3.1,

Table 1. Carbamates Determined by EPA Method 531.1 (1)

Analyte	Chemical Abstract Services Registry Number/
Aldicarb (Temik)	116-06-3
Aldicarb Sulfone	1646-88-4
Aldicarb Sulfoxide	1646-87-3
Propoxur (Baygon)	114-26-1
Carbaryl (Sevin)	63-25-2
Carbofuran (Furadan)	1563-66-2
3-Hydroxycarbofuran	16655-82-6
Methiocarb	2032-65-7
Methomyl	16752-65-7
Oxamyl	23135-22-0

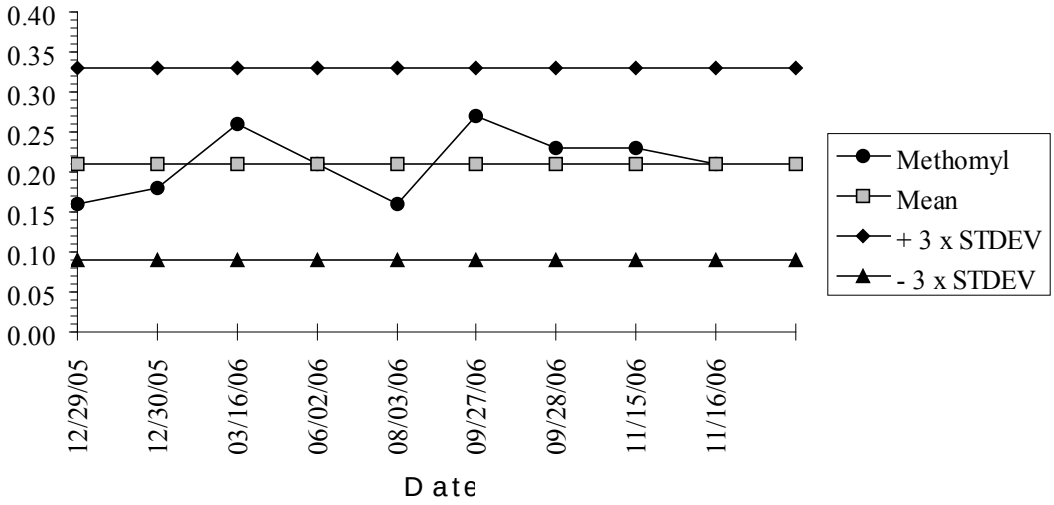
Table 2. Pinnacle PCX Trial: EPA Method 531.1 Carbamate Method Detection Level Study*

DATE	DATA SOURCE	ALDICARB SULFOXIDE µg / L	ALDICARB SULFONE µg / L	OXAMYL µg / L	METHOMYL µg / L	3-HYDROXY CARBOFURAN µg / L	ALDICARB µg / L	PROPOXUR µg / L	CARBO-FURAN µg / L	CARBARYL µg / L	METHIO-CARB µg / L
01-31-06	LFB1	1.93	1.96	1.92	2.09	1.99	2.09	2.16	1.97	2.06	2.03
03-15-06	LFB2	2.03	1.92	2.00	1.91	1.98	2.08	1.89	1.94	1.87	2.10
04-26-06	LFB3	1.97	1.99	2.07	2.08	2.05	2.13	2.01	2.01	2.06	2.08
07-01-06	LFB4	2.06	2.13	2.03	2.06	1.96	2.01	1.88	1.97	2.16	2.27
08-01-06	LFB5	2.03	1.90	2.05	2.02	2.01	2.12	2.11	1.98	2.06	2.07
09-28-06	LFB6	1.95	1.97	2.08	2.03	2.06	1.96	1.94	1.99	2.06	2.16
11-15-06	LFB7	2.00	2.00	2.09	2.13	1.89	2.01	1.99	2.07	2.00	2.12
AVERAGE		2.00	1.98	2.03	2.05	1.99	2.06	2.00	1.99	2.04	2.12
STD DEV		0.05	0.07	0.06	0.07	0.06	0.06	0.11	0.04	0.08	0.08
MDL	t* σ	0.15	0.23	0.19	0.22	0.18	0.20	0.33	0.13	0.26	0.28
LCL	0.64MDL	0.10	0.15	0.12	0.14	0.12	0.13	0.21	0.08	0.16	0.16
UCL	2.20MDL	0.33	0.51	0.42	0.48	0.40	0.44	0.73	0.29	0.57	0.55
MCL		4.00	2.00	200.0			3.00		40.00		
Detection limits per 40CFR141.24		0.50		2.00			0.50		0.90		

**MDL study was performed according to the procedure described in 40CFR Ch.1 (7-1-01 Edition), PT.136, App. B, pp 310-313, carbamate fortification level: 2 µg/L, Sample injection volume: 30 uL, Agilent LC1100-FLD/Pickering Pinnacle PCX Post-Column Reactor. LCL and UCL are the lower and upper 95% confidence limits based on seven aliquots;* $t_{(n-1,\ 1-a\ =\ 0.99)} = 3.143$ *for seven replicates. MCL denotes maximum contamination level. File Date: 12-28-06, Analyst: Jim Balk.*

Figure 1. Pinnacle PCX Typical Quality Control Charts for 0.2 µg/L Carbamate Sample Concentrations

METHOMYL – 0.2 µg/L LLLFB QC CHART



ALDICARB SULFOXIDE – 0.2 µg/L LLLFB QC CHART

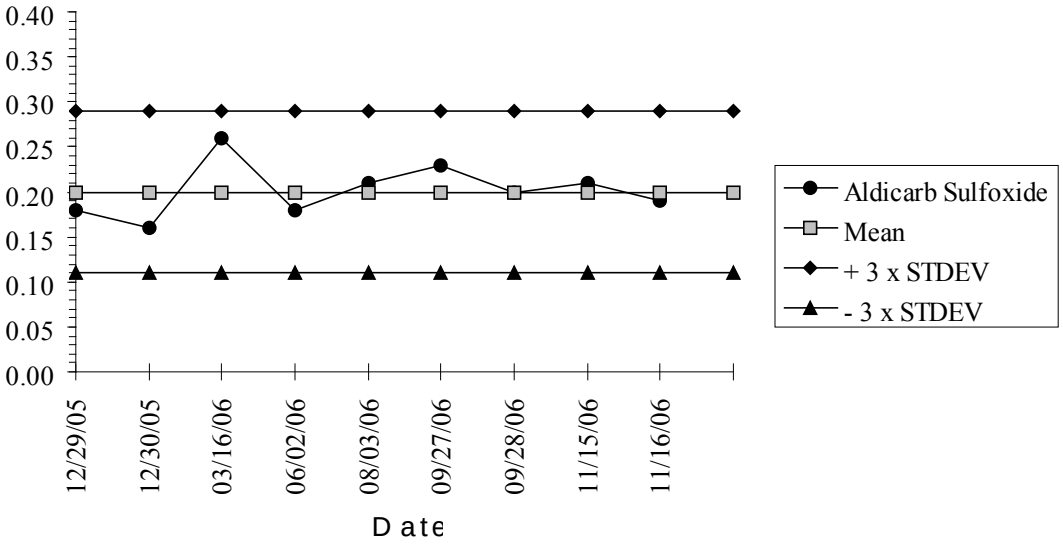
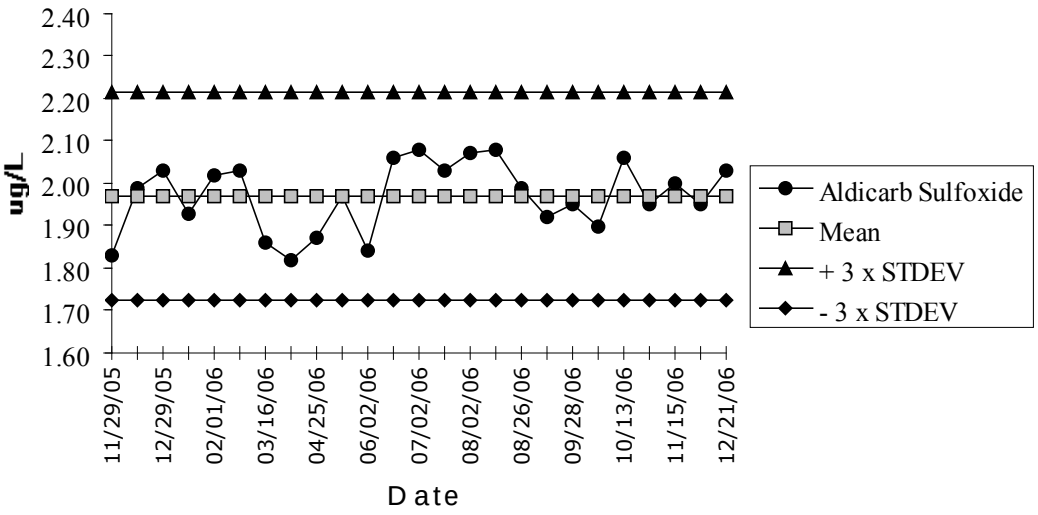


Figure 2

Figure2. Pinnacle PCX Typical Quality Control Charts for 2.0 µg/L Carbamate Sample Concentrations.

ALDICARB SULFOXIDE – 2.0 µg/L LLFB QC CHART



METHOMYL – 2.0 µg/L LLFB QC CHART