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Quality Control in the Routine Analysis of Methylmercury in Biological and Environmental Materials Using Gas Chromatography with Electron Capture Detection

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In the application of GC ECD procedures for methylmercury analysis and organomercury speciation, frequent column-treatments must be carried out to maintain the analytical system under statistical control. Often the decision to carry out a new treatment is taken without any statistical assistance. Several quality control techniques have been applied to decide the best time to make these treatments. A Trigg tracking signal chart in combination with a moving estimate of standard deviation charts gives the best indication about the timing of each new treatment.

Keywords: Mercury, methylmercury, analysis, quality control, gas chromatography, statistical methods

INTRODUCTION

Mercury is a toxic substance which is widely distributed in the environment. Organomercury compounds are much more toxic than elemental mercury or its inorganic salts. Among its organic forms, the alkylmercurials and methylmercury in particular are more toxic than the arylmercurials. Thus, the present tendency is toward the speciation of mercury and preferably, toward the determination of methylmercury present in environmental samples.[1-4]

Over several decades numerous analytical procedures have been proposed to deal with this problem, ranging from titrimetry[5] and thin-layer chromatography[6] to inductively coupled plasma mass spectrometry,[7-9] and including absorption and fluorescence (cold vapour) spectrometric techniques,[10-15] and emission microwave-induced plasma,[16-20] associated, in most instances, with an earlier stage of chromatogaphic separation or clean-up, allowing for speciation. High-performance liquid chromatography has also been used[21-25] and, very recently, capillary electrophoresis.[26] However, the most common technique being used for routine analysis in environmental studies and production quality control is the separation by gas chromatography with species detection using electron capture detection (GC ECD).[27-41] Many of these procedures are based on the classical studies by Westöö[27] using packed or, more recently, capillary columns.[42-45] These methods could be justified by the easy availability of the necessary instrumentation for most modern laboratories and the relative simplicity of the operational procedure.

However, several of the researchers have pointed out difficulties associated with the chromatographic system (e.g., low response to methyl- and ethyl-mercury due to interactions with the column and/or decomposition; peak tailing; low efficiency of the column; variable decrease in the areas and heights of organomercury peaks when injected with extracts of fish and sediment). Most authors, whether using packed or capillary columns, have stressed the need to carry out pretreatment of the stationary phase, which generally entails the injection of large amounts of mercury(II) chloride or iodide at regular intervals.[46-48] Other authors prefer derivatizing organomercurials in order to avoid such pretreatments.[49-51] Rubi[52] and Petersen[53] have recently shown the influence of stationary-phase thickness in the necessity, efficiency and duration of mercury(II) chloride treatments; they reached the conclusion that 5 μm phase-thickness columns

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do not need those pretreatments when used for the first time and can be used satisfactorily during periods of weeks or even months depending on workload. A phase thickness of 5 μm has been the choice but despite this, these columns need to have pretreatments after a certain running time. From the studies of Rubi *et al.*[52] it is clear that the pretreatments enable the use of the columns for longer periods of work but at the same time destroy stationary-phase homogeneity. The consequence is that as the column is used more and more, it becomes necessary to carry out treatments at more frequent intervals because the efficiency and duration of effectiveness of the treatment decreases. At the end of the column lifetime the treatments barely last long enough to carry out the calibration injections; that is a clear symptom that the column must be substituted by a new one.

Of course, in routine analysis of methylmercury one must consider the cost of the column as a part of the complete analysis cost, and assume that the column would be able to run a limited number of analyses, needing from time to time the above-mentioned treatments to produce reliable results. But the problem is how to decide that the moment for a new treatment has come. If excessively frequent treatments are carried out the lifetime of the column will decrease accordingly. But on the contrary, unrealistic results would be obtained if the treatment were not carried out when needed. Often this decision is taken by the chromatographer based on experience and intuition on seeing the shape of the methylmercury peaks obtained. Obviously a quality control policy for the laboratory work would aid this decision. In this paper a study of the use of quality control charts to decide the moment for the treatments is presented.

EXPERIMENTAL

Reagents

All the reagents and solvents were of the highest purity commercially available. Working and calibration solutions were prepared by dilution in toluene of a 1.0000 g^{-1} methylmercury chloride stock solution. These solutions were kept refrigerated and protected from light. The column treatment solution was a 1% mercury(II) chloride in toluene.

Apparatus

All the experiments were carried out with a Hewlett–Packard model 5890 Series II gas chromatograph equipped with a nickel-63 electron-capture detector using N55 nitrogen as carrier and makeup gas. Samples were injected by means of a Hewlett–Packard model 7673 automatic injector. A Hewlett–Packard model 3396A integrator was used for data acquisition and reprocessing. All the samples were run on the same capillary column, an SGE BP-1 (dimethylsiloxane) 25 m long and of 0.53 mm i.d. fused silica with 1.0 μm phase thickness). Statistics and quality control charts were obtained by means of Statgraphics plus V.6.0 (Manugistics Inc.)

Column treatments

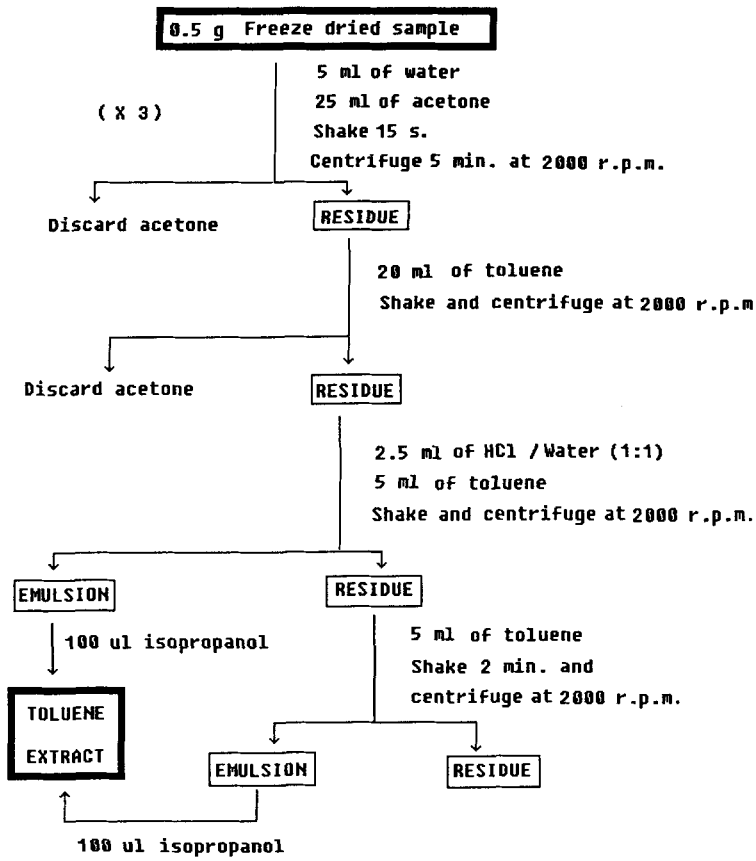
The column treatment process basically followed O'Reilly's procedure,[46] except for the temperature, and has been described elsewhere.[52] For low- or medium-polarity columns as used in this work, five injections of 10 μl mercury chloride treatment solution at 5–10 min intervals, maintaining the oven at 115 °C, allowed a complete treatment of the column.

Samples and operating procedures

Two types of samples were analyzed. Samples were obtained during an intercomparison exercise of the European Community Bureau of Reference within BCR-Program RM-311. Additional amounts of these samples were kindly submitted for this long-term study by other participants in the same exercise, thus allowing more than 100 analyses of each material. All the analyses were carried out in duplicate following the procedure described by Hight and Corcoran[37] with minor changes described elsewhere.[52] A diagram of this procedure is shown in Scheme 1. At the time of the study described in this paper, this procedure had been in use at our laboratory for several years and all the analysts participating had a long experience in the operating steps.

Calibration

Calibrations were done at four levels ranging from 10 to 40 ng ml^{-1} methylmercury in toluene, showing typical regression coefficients of 0.998. During the time covered by the results described in this paper, the chromatographic system was recalibrated at regular intervals (two days) and



Scheme 1 Schematic diagram of the analytical procedure used for methylmercury determination.

after each new column treatment. Standards were also included routinely between samples to validate the calibration line currently in use.

RESULTS AND DISCUSSION

Sample I was a freeze-dried and homogenized tuna muscle with a high content of methylmercury. Sample II was a freeze-dried and homogenized mussel tissue with a low content of methylmercury. Over three months, portions of both materials were analyzed daily within the normal schedule of the laboratory. This means that other samples were currently being analyzed each day for methylmercury (mainly mussels) following other studies in which the laboratory was involved at that time. This fact has to be stressed to understand the frequency of the column treatments, which cannot be justified by the number of chromatographic runs referred to in this paper, and also to understand that the treatments were not carried out on a periodical time basis.

Data accumulated during the first three days of analysis (18 runs) were used for the establishment of a standard Shewhart X-control chart for each material with the parameters summarized in Table 1. Normality tests and Box–Whisker plots showed acceptable results, so the data were considered adequate to the initial chart setup. From this moment the X-Chart was used to control day-

Table 1 Statistical parameters of the data used for the establishment of control charts

	Material I: tuna muscle	Material II: mussel tissue
Mean	3.92 $\mu g\,g^{-1}$	0.19 $\mu g\,g^{-1}$
Std deviation	0.41 $\mu g\,g^{-1}$	0.03 $\mu g\,g^{-1}$
Median	3.83 $\mu g\,g^{-1}$	0.19 $\mu g\,g^{-1}$
Mode	3.60 $\mu g\,g^{-1}$	0.17 $\mu g\,g^{-1}$
Std skewness	0.34	0.36
Std kurtosis	−0.78	−1.09

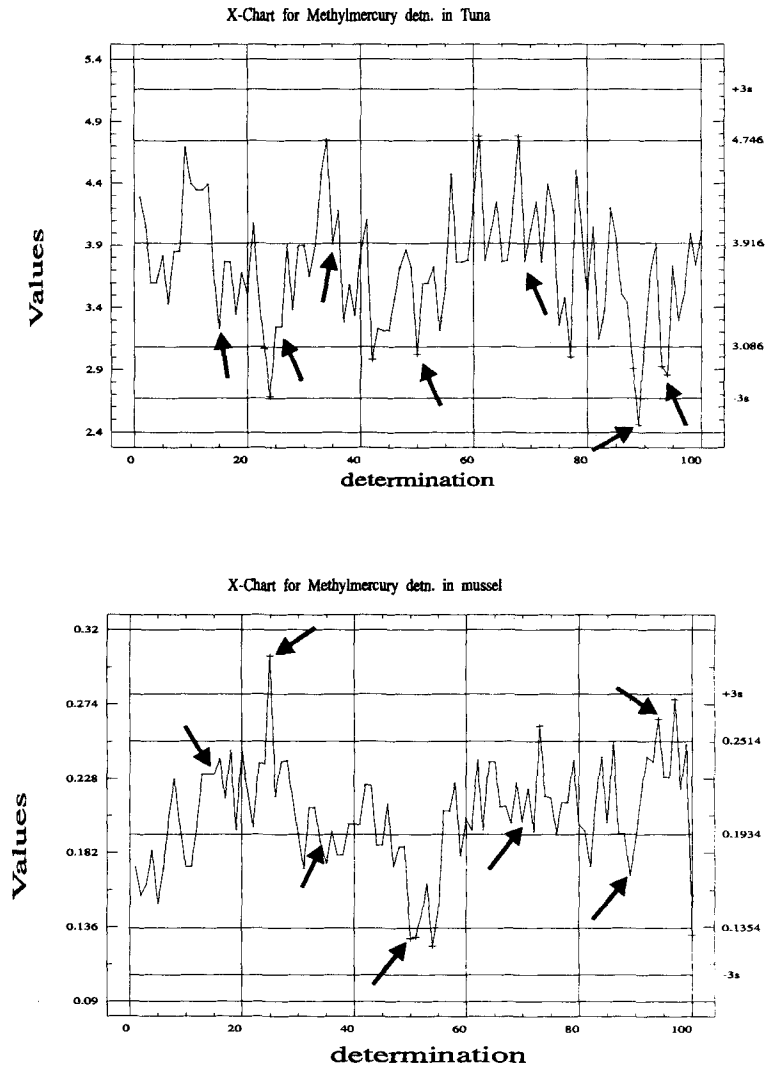


Figure 1 Shewhart Control Charts for the materials analyzed (arrows indicate first point after each new column treatment).

by-day analyses. Operating characteristic curves for the established X-Charts show that relatively large deviations (up to 30% with respect to the corresponding means) could be undetected by the charts. This is not surprising in view of the standard deviation data in Table 1 and is to be accepted in this type of analytical procedure, involving several cleanup, extraction and back-extraction stages. When out-of-limits points were observed it was decided to carry out a new column treatment. This decision was helped by the appearance of the methylmercury peak in the injections of standards made between samples—this tends to broaden and become asymmetric when the treatment is ending its efficiency time. Charts in Fig. 1 show the data acquired for both materials. In these charts the arrows indicate the days where a new column treatment was carried out. It can be seen in these graphs that only one point in each chart drops out of the external control limits (±3 SD) and few points drop out of the internal (±2 SD) control limits. Thus, a first look at the control charts would induce the conclusion that the system was under control most of the time. Obviously, column treatment is not the only factor to take account, since sample preparation involves several cleanup and extraction stages. Outliers could be attributed to failures in any of these stages and it is assumed that the treatments have been carried out at the right time to maintain the analytical system under statistical control. Thus, in this type of analytical process

one can distinguish between two kinds of effects causing out-of-control points: (i) occasional errors in any of the process stages, and (ii) trends which modify the chromatographic response, changing the process mean. However, it is well known that Shewhart X-Charts reveal the presence of out-of-control points once the system has been derived, but lack the ability to inform us about possible shifts of the system or trends that would have out-of-control consequences. On the other hand, a good-quality control policy implies detection of system trends well before out-of-control points should appear. This means that Shewhart X-Charts, although they are most usual quality control charts in the analytical laboratory, would not be suitable in the particular case of methylmercury analysis.

For shifts and trend detection there are well-suited statistical tools that have been described for many years and are routinely used in between-batch quality control.[54] In fact, the problem we are dealing with can be modeled as a between-batch quality control study, assuming that our analytical system produces each day a batch of results by means of the application of a specific methodology. So the problem is to find out if our system runs every day under statistical control giving target average quality results. For these situations, the more common technique is the CuSum Chart.[54,56] Figure 2 shows some CuSum charting results for the study of Materials I and II. Figure 2 graphs correspond to the detection of the first out-of-control trend. The CuSum V-Mask was constructed to detect shifts over ± 1 SD about the target mean (see Table 1), assuming alpha and beta risks of 0.05. It can be seen that the chart detects this trend at point 23 of the measurement series and the shift is clear at point 24. The result is the same for both control materials, irrespective of methylmercury concentration level and the fact that the trend shows opposite tendencies for tuna (where the shift started at point 13) and mussel (where shift started at point 12) samples. This means that the shift started before the initial chart establishment and just before the second column treatment. In Figure 1 it can be appreciated that the first out-of-control (inner control limit) appears at point 24 for tuna and at point 25 for mussel, suggesting that the column treatment is necessary.

Several CuSum-derived charts have been proposed for between-batch quality control studies. One of them, the tracking signal proposed by Trigg[55] and described in detail by Hartley,[56] is a tracking signal, derived from the CuSum, obtained by dividing a smoothed estimate of the CuSum (named the smoothed error) by the mean absolute deviation (MAD). The use of the smoothed error instead of the true CuSum signal restrains the tracking signal obtained to limits of ± 1, thus correcting some of the drawbacks of previous proposals.[57] The graphs shown in Fig. 3 correspond to the Trigg tracking signal for the materials studied. Specific values of the CuSum V-mask alpha dimension were obtained to be sure of detecting shifts equal to or more than 10% over the target mean. The actual values of the signal were obtained by implementing the BASIC listings given by Hartley.[56] These listings must be corrected for typing errors in lines 3310, 3398, 4196 and 4202, and changes in line 3614 to obtain a more accurate estimation of the maximum and minimum values of the time series. The programs listed by Hartley produce only a graphical output of the X-Chart and write the numerical values of the Trigg tracking signal, the CuSum V-mask process mean and the moving estimate of the standard deviation. We have introduced routines in the Hartley listings to obtain all this relevant information in ASCII files that can be processed by other packages (e.g. STATGRAPHICS plus) to obtain good-quality graphs and help in interpretation (listings of the amended Hartley programmes may be obtained from the authors on request).

When an alpha value of 0.2 is used to obtain the Trigg tracking signal (Fig. 3), the signal responds to a sustained drift within the first two or three data points. The graph for tuna muscle in Fig. 3 shows clearly that the trend was influencing the results when the column treatments were carried out, but fall immediately down to limits after the control action (treatment). This means that the treatment, in fact, was applied too late (out-of-control points occurred in the Shewhart X-Chart at this moment) but the control action was effective (the system falls down to the control limits because of the treatment). The presence of two outliers (probably caused by accidental errors in the sample preparation stages) is also clearly stressed by the graph. In contrast, for mussel material the Trigg tracking chart suggests that the treatments have apparently been carried out at the right time but the system continues to produce out-of-control results during some runs. A possible explanation of these contradictory results can be obtained from the graphs of the moving estimate of the SD[54,56] shown in Fig. 4. Although

precision of the measurements was not good in any of the materials tested, the graphs show a worse situation for mussel material. This is because for low methylmercury content materials (such as the mussel material analyzed here), detector signals are close to the detection limit and another factor has to be taken into account. This factor is the time the column needs to recover a clean baseline after mercury(II) treatments. During mercury(II) treatments the column is disconnected from the detector port. This is essential to avoid serious detector contamination. After several hours (12–16 h have been the choice in our laboratory) the column is connected to the detector to wait for a flat baseline. Usually this process was monitored by injecting toluene blanks and measuring any residual signal in the retention time window corresponding to methylmercury. When no signal was apparent in the chromatograms, routine analytical work started. The Trigg tracking chart for mussel material probably suggests that the column generally was not so clean as was believed. This fact could justify results that continue the upward trend after the

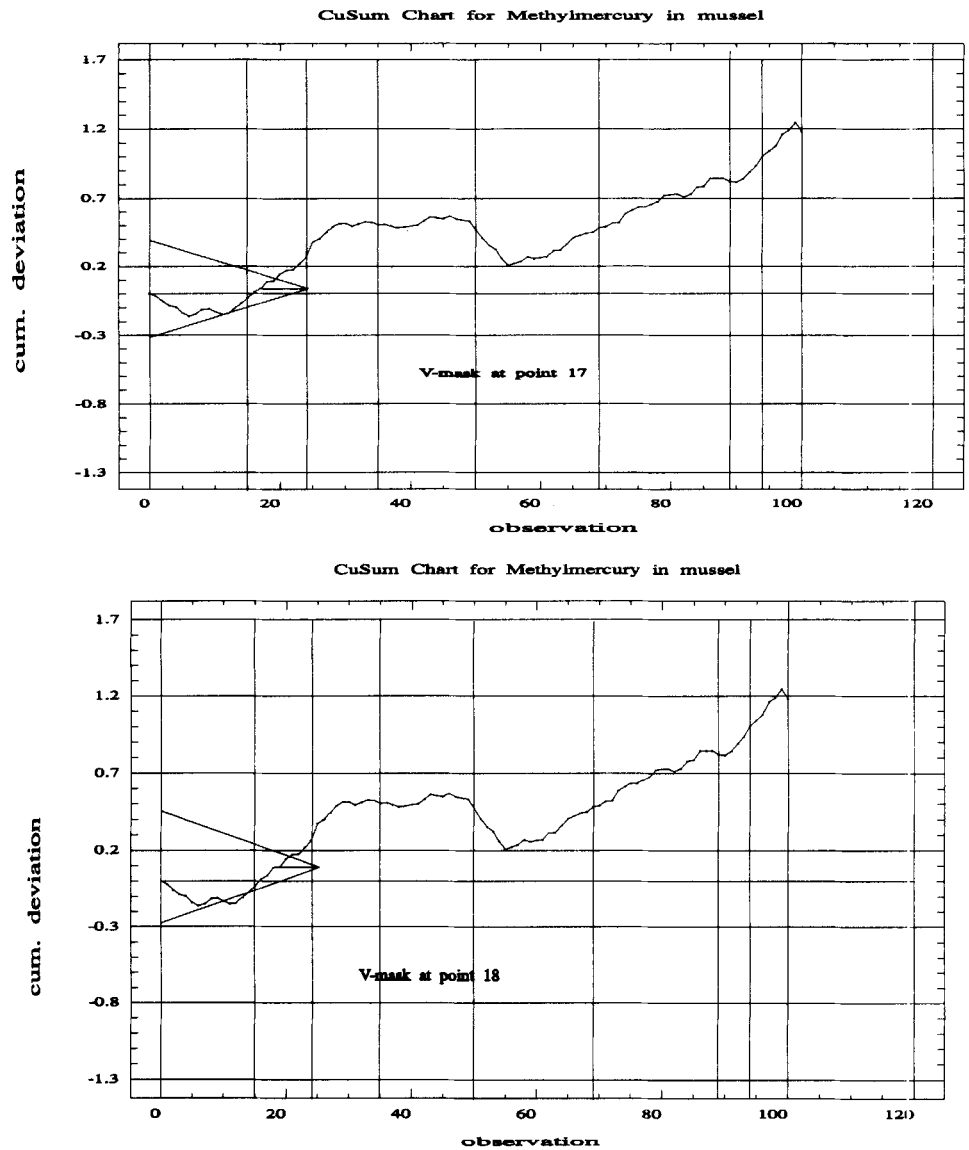
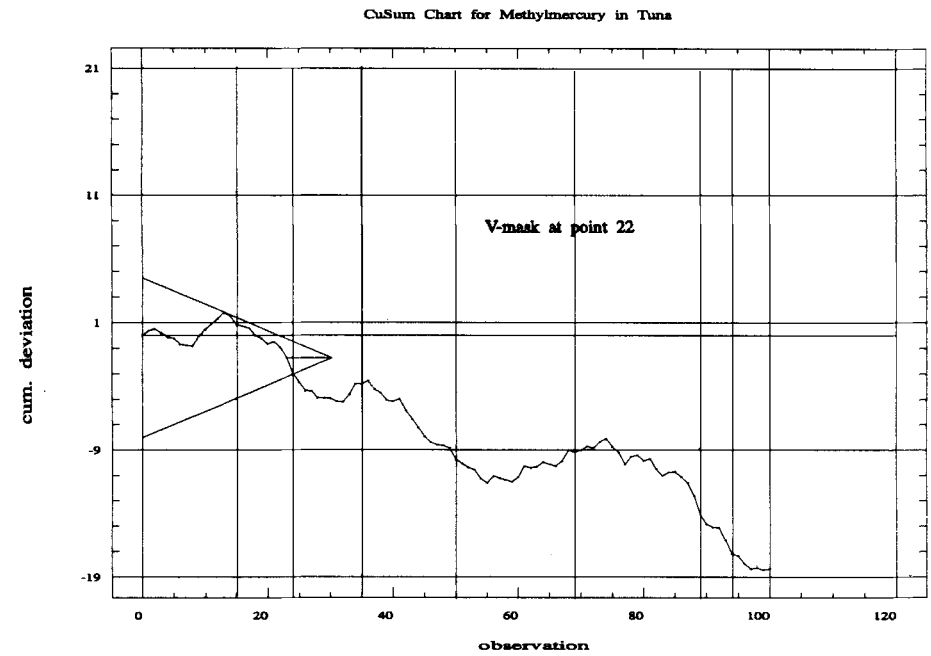


Figure 2(a)



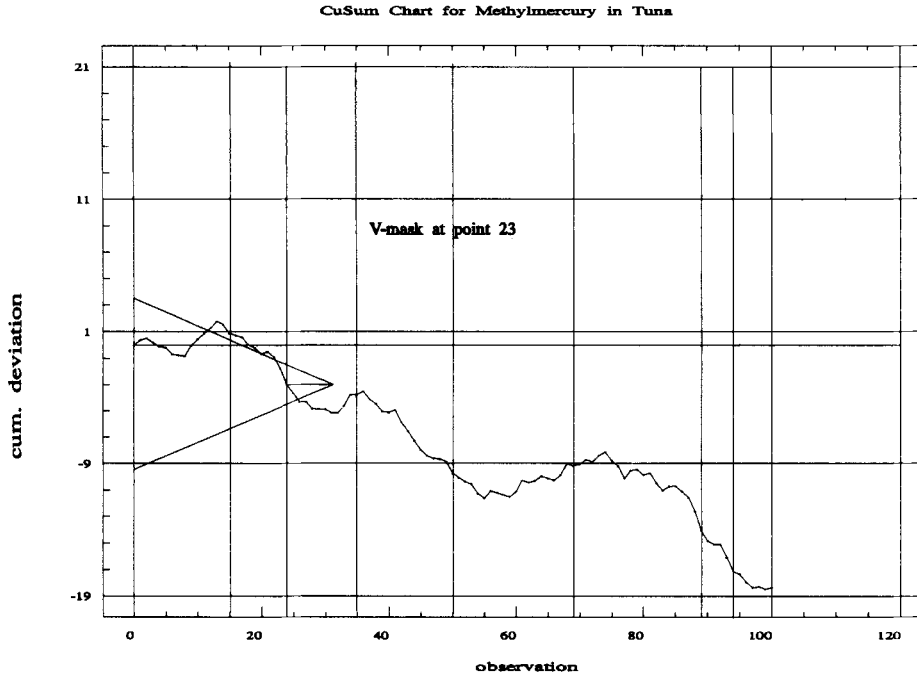


Figure 2(b)

Figure 2 CuSum Charts for the detection of the first out-of-control trend. (a) Tuna muscle, V-mask at points 22 and 23. (b) Mussel tissue, V-mask at points 17 and 18. (Vertical lines on the chart match with each new column treatment.)

treatment. A similar suggestion can be obtained from the trends detected in the CuSum graphs in Fig. 2. Obviously, for high methylmercury content materials (like the tuna muscle analyzed), this influence cannot be appreciated because errors due to treatment residues are well within

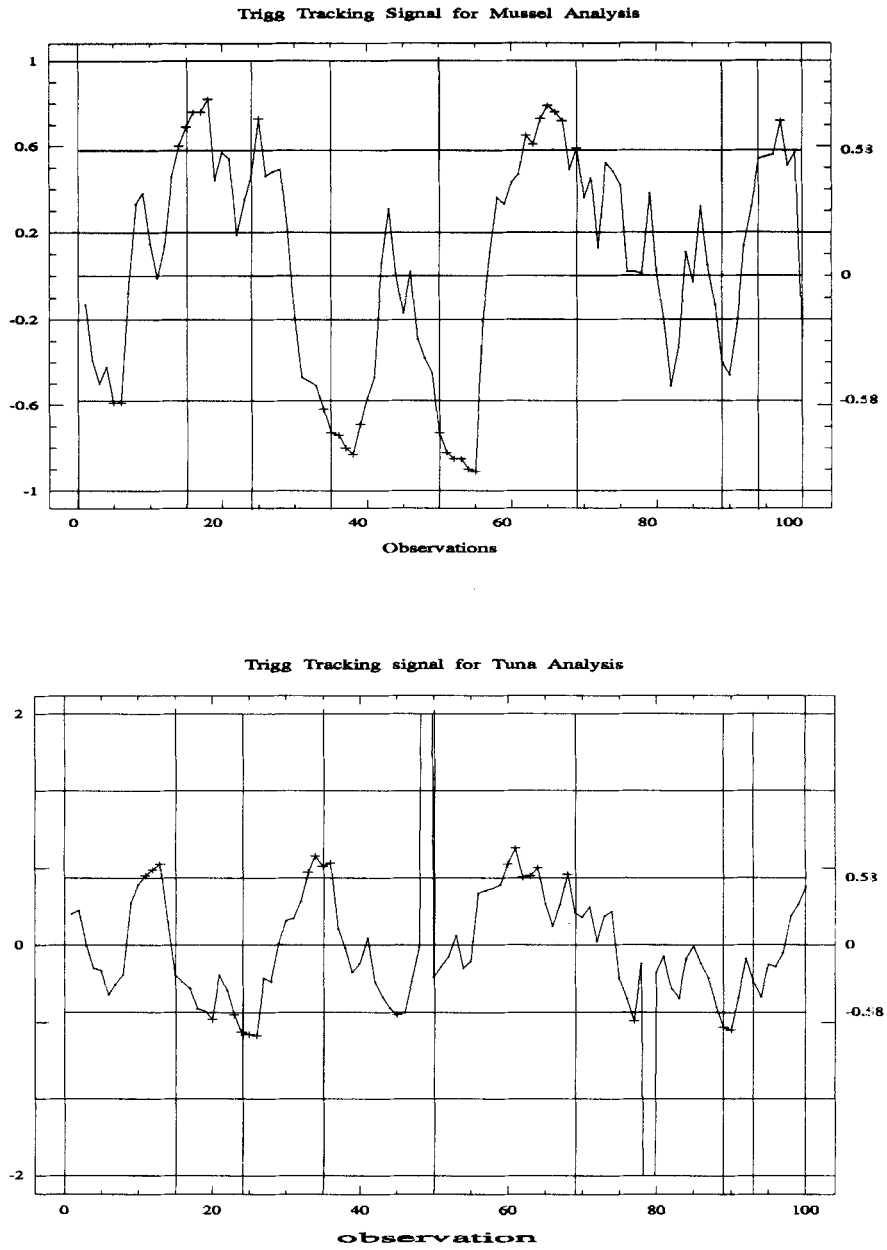
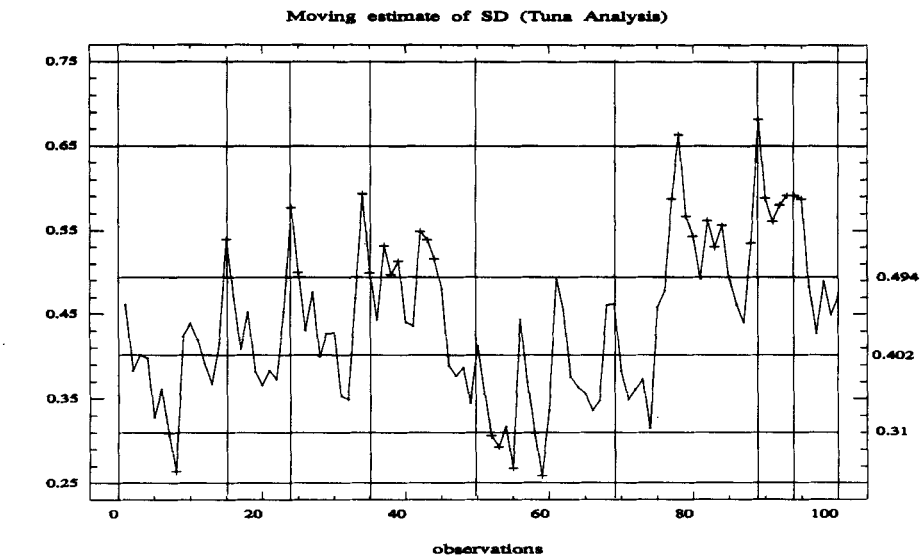


Figure 3 Trigg tracking signal plots for the materials studied. For the conditions of chart build-up, see text. (Vertical lines on the chart match with each new column treatment.)

the experimental variability. On the other hand, for tuna there appears a region between points 78 and 100 of the very poor precision where treatments apparently cannot eliminate this behavior. At this time the column has processed several hundred injections and is reaching the end of its lifetime. The Trigg tracking signal is not able to detect poor precision situations efficiently, this behavior being typical of this tracking signal.[56]

It is important to appreciate that these types of chart (Shewart-X, CuSum and/or Trigg tracking charts) have to be used in combination and that the information given by each one must be carefully balanced with the others. The moving estimate of the SD chart provides a reliable local estimate of the SD of the QC data, thereby reflect-



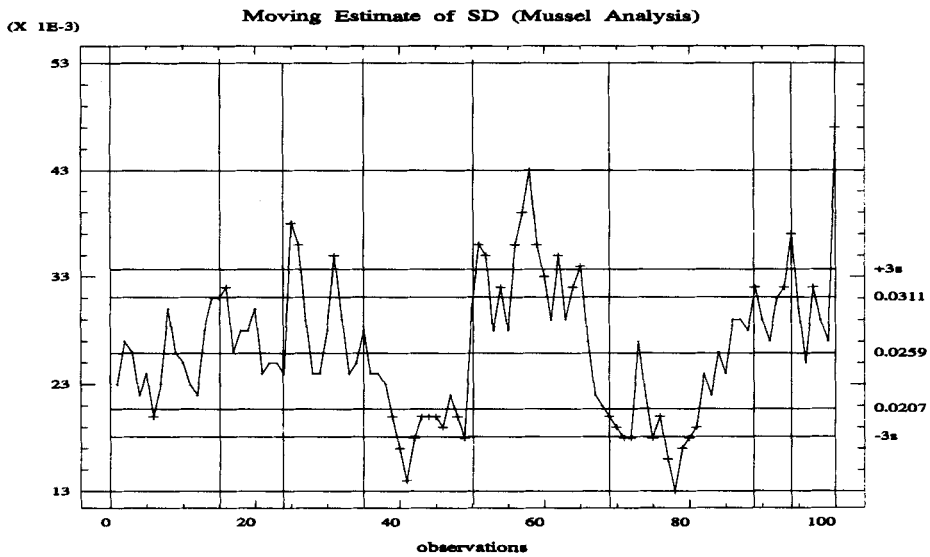


Figure 4 Moving estimates of the standard deviation for the materials studied. (Vertical lines on the chart match with each new column treatment.)

ing the current precision of the analytical method when used routinely. The other conclusion is that the use of the Trigg tracking signal could suggest the right moment to carry out the column treatments well before the analytical system goes out of control. Moreover, the use of the Trigg tracking signal charts has the practical advantage compared with the CuSum charts that all the signal is contained within the same window of values of ± 1, making easier data handling.

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