



## A Multivariate Approach to Monitor the Quality of Chromatograms from Gas Chromatography Mass Spectrometry

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Multivariate control charts were generated to accurately track and monitor data produced from large batches of samples in continuous runs. The Shewhart and cumulative sum (CUSUM) charts were evaluated for their ability to determine the quality and stability of chromatograms produced from control sample runs on GC-MS. The data generated from continuous run processes were statistically analyzed in order to evaluate the quality of measurements obtained. A sample of a GC-MS continuous data run is presented to illustrate the effectiveness of these multivariate control charts to monitor quality of data output. Moreover, the statistical analysis of variance (ANOVA) technique was able to identify the specific factors that significantly contribute to GC/MS instrument data output quality. In conclusion, this chart-based approach to quality control, when applied to ongoing GC-MS processes, will enhance the overall data output quality and improve the detection effectiveness.

**Key Words:** Quality control chart, Shewhart control chart, Cumulative sum control chart, GC/MS.

### INTRODUCTION

It is critical to continuously monitor the output quality of data from long duration runs of large sample groups from any automated instrument. In particular, analysis of volatile compounds can produce thousands of signals that are applied to downstream calculations, which in turn are used to guide automated programs that determine the variance and stability of the samples under investigation<sup>1-3</sup>. For example, in the modern-day food industry, food and food storage units are tested for their ability to maintain safe temperatures (forestalling spoilage that can otherwise result in lost monetary value or detrimental effects on human health). Extent of and duration to food spoilage can be detected by subtle variances in volatile compounds. Thus it is critical that the instruments used to detect these compounds are highly sensitive and accurate. Moreover, if the automated analytical system operates continuously, the output measurements need to be checked throughout the operation process and preferably, in real-time to readily recognize and correct low quality data. Such quality control methods have been developed and are commonplace in large scale business operations<sup>4</sup>. The most simple and direct method is by performing statistical estimations and investigating any data that deviates from the expected profile<sup>5</sup>. Using graphical plots of data allows for a visual display of quality characteristics over a period of

time and facilitates easy identification of measurements that deviate from the control charts<sup>6</sup>. However, this procedure can rely on at least some judgement based on expert opinion. Quality control (reference) samples are required in the analytical experiments and they must be a highly accurate representative of the sample<sup>7-9</sup>. A reference sample must fit the following criteria: (1) relatively stable quality over an extended period of time; (2) produce quantitative measurements nearly identical to the analytes of real samples; (3) have a basic composition similar to the real sample.

The cumulative sum (CUSUM) control chart was originally developed in 1954<sup>10</sup> and is able to accurately detect small changes in analyte data by making use of information from observations collected prior to the most recent data point<sup>11,12</sup>. This chart works by conforming serial measurements to determine variances experienced during the overall analysis process. A mast in the shape of a V is usually laid over the chart with an origin over the last plotted point<sup>13</sup>. Previous points covered by the mask indicate the process was shifted. Unfortunately, the cumulative sum chart is limited by the fact that it is not fixed or parallel. On the other hand, the Shewhart control chart was developed in 1993 for the purpose of monitoring continuous quality of analyte data output<sup>14</sup>. However, the Shewhart chart is limited in its ability to efficiently detect small changes, such as a 1- or 2-sigma change in the mean.

We sought to investigate the applicability of both of these two charts to monitor quality in real-time of sample data produced by GC-MS. A total of 13 musk compounds kept in-house and having stable chemical character were used as the samples in this study. The flowchart of the process of the calculating of the normal operating conditions region is shown in Fig. 1.

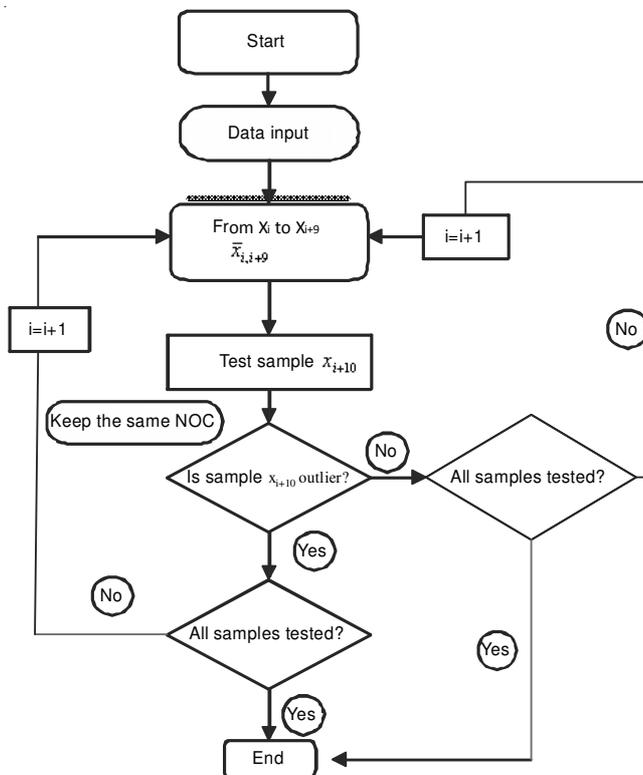


Fig. 1. Flowchart of the process of calculating the normal operating conditions region

## EXPERIMENTAL

The reference compounds used in this study were: musk xylene (MX), musk ketone (MK), musk moskene (MM), galaxolide (HHCB), tonalide (AHTN), celestolide (ADBI), phantolide (AHMI), traseolide, galaxolidone (HHCB-lactone, the metabolite of galaxolide) and triclosan (all kindly donated by Dr. Jean-Daniel Berset Berset (Office of Water and Waste, Switzerland); MX-d15 (purchased from Dr. Ehrenstorfer, GmbH, Germany); and ONTE (purity 98%; purchased from Toronto Research Chemicals Inc., Canada). Stock solutions (100 mg/L) were prepared by dissolving in isooctane and solutions stored at  $-18\text{ }^{\circ}\text{C}$  until use in GC/MS analysis.

**Instrumental parameters:** A micromass quattro micro GC tandem mass spectrometer (Waters, USA) and a 6890 gas chromatographer equipped with a  $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$  DB-5MS capillary column (Agilent Technologies, USA) were used. A  $1\text{ }\mu\text{L}$  sample was injected by the splitless method at  $280\text{ }^{\circ}\text{C}$ . Following a holding time of 0.5 min, the GC oven temperature program was initiated: from  $70\text{ }^{\circ}\text{C}$  to  $180\text{ }^{\circ}\text{C}$  at a rate of  $20\text{ }^{\circ}\text{C}/\text{min}$ , followed by an increase to  $220\text{ }^{\circ}\text{C}$  at  $2\text{ }^{\circ}\text{C}/\text{min}$ , then to  $30\text{ }^{\circ}\text{C}/\text{min}$  until  $280\text{ }^{\circ}\text{C}$  was achieved when a final hold time of 2 min was carried out. Helium was used as the carrier and applied at a constant flow rate of  $1.0\text{ mL}/\text{min}$ . A total of 11 functions were obtained by the multiple reaction moni-

toring (MRM) modes and used to identify the target chemicals (Table-1).

TABLE-1  
THIRTEEN TARGET COMPOUNDS THAT  
WERE MONITORED CONTINUOUSLY

Name	Chemical compound
ADBI	Celestolide
AHMI	Phantolide
MA	Musk ambrette
HHCB	Galaxolide
AHTN	Tonalide
MX	Musk xylene
MK	Musk ketone
MM	Musk moskene
MT	Musk tibetene
HHCB-lactone	Galaxolidone
d15 MX	Musk xylene D15
OTNE	Isocyclemone
D10-phe	None

**Parameters preparation:** The values of four parameters [peak height, retention time (RT), peak areas and noise factors (NF)] were recorded over a one month period from 65 standard mixture samples. Each reference sample contained a series of known volatile compounds and was run daily on the GC and MS instruments. The peak height, peak area and retention time were calculated by Masslynx software (Waters). The program for identifying the peaks was able to be manually edited and adjusted from the default method. A sample of one of the full total ion chromatograms (TIC) obtained is presented in Fig. 2. Using the multiple reaction monitoring mode, the peaks were more easily extracted from the total ion chromatograms plot and the parameters were much more precise. The results were compiled in a daily report that was sent back to the analysts and used to assess how well the instrument was performing and whether samples were of acceptable quality. In some cases, actions, such as cleaning the EI probe or changing the column, were deemed necessary prior to further acquisition of samples.

**Software:** Masslynx software was used to extract the target peaks from total ion chromatograms. All subsequent data analysis was performed with MATLAB (version 7, release 14). The analysis of variance (ANOVA) tool was used and  $P < 0.05$  was considered statistically significant.

## Data analysis

**Measurements of parameters in control charts:** Retention time, noise factors, peak heights and peak areas were measured for all 13 compounds by using the Shewhart chart and cumulative sum chart. Since the numbers for peak height and peak area were large, they have been presented in table format after scaling (Table-2). The data that comprises Table-2 is a randomly selected sampling to show how various measurements in the daily report were calculated.

The peak heights and peak retention times were extracted by manual inspection of the characteristic single ion chromatograms (SIC) for each compound. It is important to note that the peak heights were not calculated by the total ion chromatograms, which would give greater values than the peak height calculated from the single ion chromatograms. The peak in the total ion chromatograms tended to have a higher baseline

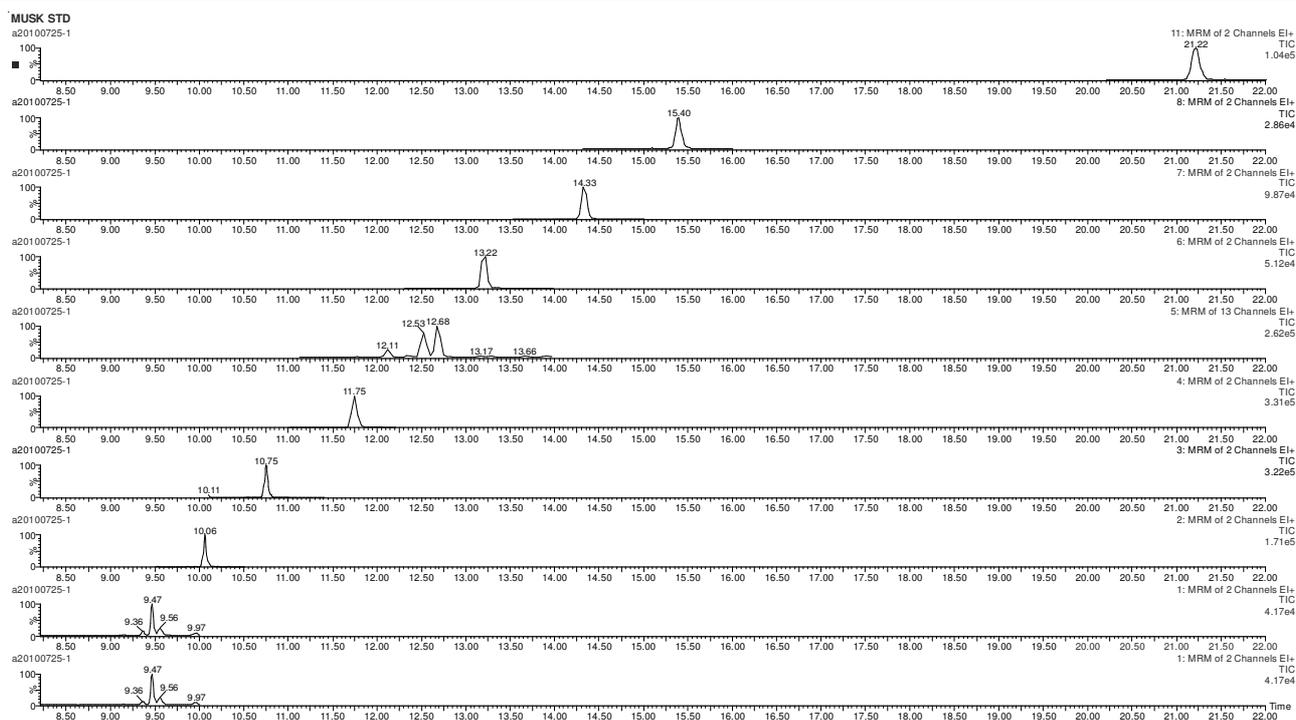


Fig. 2. Full image of total target chemicals

and single peaks sometimes overlapped with other peaks; thus, the extracted peak height from total ion chromatograms would have not yielded a sufficiently representative value.

TABLE-2  
SAMPLE MEASUREMENTS FOR PARAMETERS

Compound	Precursor ions	Peak height	Peak area	RT (min)	NF
ADBI	244.4	31544	0.076	9.96	4.19
AHMI	244.0	28912	0.579	10.62	4.51
MA	268.3	19496	10.167	11.96	5.75
HHCB	258.4	23384	10.004	12.41	7.37
AHTN	258.4	22536	1.474	12.56	5.07
MX	297.3	20352	1.697	12.45	5.58
MK	294.3	22344	0.822	15.20	6.04
MM	278.3	28672	2.591	13.03	5.39
MT	266.3	19224	0.151	14.15	5.88
HHCB-lactone	272.4	31992	0.107	20.99	5.98
d15 MX	242.1	31544	0.298	12.19	5.90
OTNE	234.4	28912	0.199	9.38	5.96
D10-phe	289.5	19496	2.118	11.56	5.753

\*Values less than 10 are considered acceptable.

The peak area calculation was carried out by using the automatic peak detection of masslynx. Peak areas for each of the 13 compounds under investigation were transformed to the MATLAB platform<sup>15</sup>. The parameters in MarkerLynx™ were set as follows: peak width, 5 %; height, 12 s; peak-to-peak baseline, 75; intensity threshold, 85 counts; mass window, 0.02 Da; mass tolerance, 0.05 Da; retention time window, 0.1 min; noise elimination level, 4. The period of retention time was processed from the beginning of the chromatogram at 0.1 min to its end at 25 min. Two or three common peaks needed to be checked manually in the aligned peak list; this facilitated

the main parameters being set as a properly defined window in which common peaks were found in every chromatogram using retention time and mass pair in the spectra. The peak area principle was calculated by summing the individual single ion chromatograms peak areas (which were grouped together) and the mass spectrum of that peak (taken as the height of all selected single ion chromatograms at that particular time point). For daily monitoring of quality control samples, this peak detection script was run on the quality control sample under investigation. By using knowledge of the approximate retention time and mass spectrum of each of the 13 compounds, we were able to determine whether the peak detection script had been able to identify each of the compounds. If the procedure had worked correctly, then the area was simply taken from the script's results. If the peak had been completely overlapped by another compound, had very low signal to noise ratio or was very broad and noisy, then the peak was considered to not have been found by our programs.

The noise factor was calculated to monitor the noise level of the systems as they were run over a long period<sup>16,17</sup>. Noise factors up to 10 was considered acceptable and the noise factors in all the previous reference samples was low. The calculated noise factors for the sample was included in both the Shewhart and cumulative sum charts. Noise factors was also used as an indicator for comparing the noise level from different instruments. Based on the reference knowledge<sup>17</sup>, the simple determination of the noise level was calculated in the following way:

$$N_f = \frac{\sigma}{\sqrt{\chi}}$$

where,  $\sigma$  is average random deviation,  $\chi$  is the detected signals in chromatograms.  $N_f$  was calibrated by the ion chromatograms of the continuous signal intensity for each chromatogram. The minimum signal to noise ratio was able to be established, representing a threshold below which a peak was deemed to

be noise. Each ion was treated separately. The single ion chromatograms was divided into segments of 13 data points in length. Any segment in which all intensities were zero was rejected. Any abundance of any segment was rejected when it was zero. According to the principle of noise factor<sup>17</sup>, the mean abundance of each accepted segment was calibrated and it was counted for the numbers of this mean value 'crossed' within the acceptable segment. The segment would be rejected that the number of crossings was less than a half of the number of scans in the segment. The mean intensity of the segment was calculated and the number of times the signal crossed the mean was also counted<sup>18,19</sup>. If the signal crossed the mean less than 6 times, it was considered to indicate a region of signal that contained information about a particular compound and was therefore not a region of noise and so was accepted. A sample obtained for our analysis of a peak at main ion 51 m/z is presented in Fig. 3. For each remaining accepted segment, the median deviation from the mean abundance for that segment was found. This deviation was divided by the square root of the mean abundance for that segment in order to obtain a sample noise factors value, which was then saved. After processing the entire ion chromatogram, the median of these noise factors values was taken as the characteristic noise factors value for this ion channel<sup>17-19</sup>. For each chromatogram in this study, 316 noise factors values were calculated and the median of these noise factors values was used as an indicator for the overall noise level measurement of the chromatogram. An example is show in Fig. 4 as the boxplot presents the noise factors value.

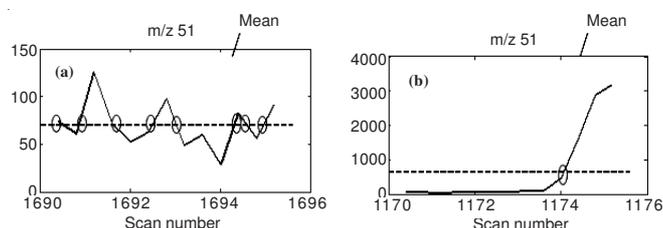


Fig. 3. Sample of the number of crossings at ion m/z 51; (a) Eight crossing points: accepted as noise; (b) One crossing point: rejected as noise

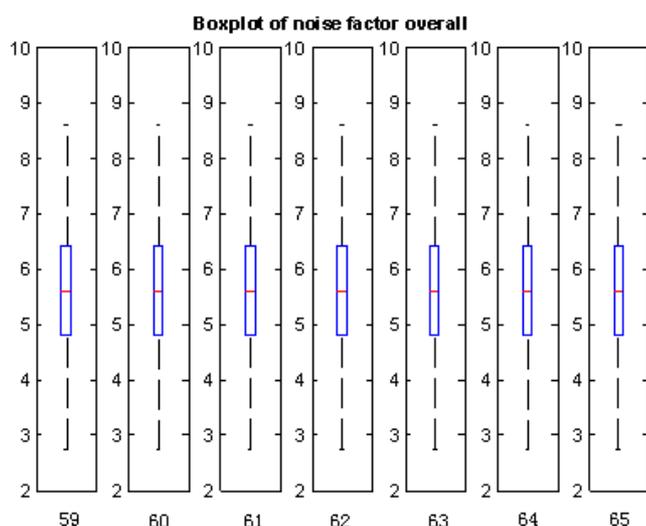


Fig. 4. Noise factors values were used as indicator for the noise level measures of the chromatograms and the boxplot showed an example of last 7 samples, which was acceptable as less than 10

**Quality control charts:** In all quality control sample processes, monitoring was carried out with the primary objective of visualizing the change in three parameters of the objective compounds.

**Shewhart control chart:** A Shewhart chart<sup>20</sup> can effectively monitor a single parameter over time and any large deviations in that parameter are visually presented. Limits can be set on the chart and the parameter monitored for times when it falls outside of these limits. Warning limits (indicating when there may be a problem that should be investigated) and action limits (when the system may need to be stopped until the fault is corrected) can be calculated and displayed on the graph by the extent of a parameter's change over time. Both of these limits are calculated based on the mean and standard deviation (s) of previous samples that are assumed to be under the operator's control, known as the normal operating conditions<sup>21</sup>.

The limits are calculated based on the mean and standard deviation of the previous samples. The mean and standard deviation were calculated from studied samples. It's considered that if the value of the parameter was normally distributed then only approximately 1 in 10 samples (based on a two-tailed normal distribution - 5 %) would be expected to fall outside of two standard deviations from the mean, while only approximately 2 in 1000 (0.2 %) would fall outside three standard deviations. The standard way to calculate these limits is to take 2 standard deviations from the mean as the warning limit and 3 standard deviations as the action limit (Fig. 5). The warning limit means, where if a sample is seen to go outside this limit, then the system should be monitored closely as maybe something is starting to change and the action limit means, where the system has changed significantly and something needs to be done. In the following equations,  $w$  denotes a sample statistic that measures a continuously varying quality, such as peak area, retention time or peak height. The mean value over a series of historic samples is represented by  $\mu_w$  and the standard deviation by  $\sigma_w$ . Thus, the centre line, the upper control limit (UCL) and the lower control limit (LCL) were calculated as follows:

$$UCL = \mu_w + k \cdot \sigma_w$$

$$\text{Centreline} = \mu_w$$

$$LCL = \mu_w - k \cdot \sigma_w$$

where,  $k$  is the distance of the control limits from the centre line, expressed in terms of standard deviation units (two for the warning and three for the action limits).  $\sigma_w$  is standard deviation of sample statistic value.

There were several methods available to calculate the mean and standard deviation: (a) using all of the previous samples; (b) using just the previous five samples; (c) using the five samples before the previous five samples.

The related Shewhart chart that was generated using information from 11 samples is shown in Fig. 6, in which the most recent sample is plotted in green on the far right. The three different types of warning lines are represented by the following colours: 1-10, blue; 6-10, green; and 1-5, red. The mean is represented by a solid line, the warning limit by a broken line (- . -) and the action limit by a dotted line. The peak areas of first 10 and last 10 samples showed that no much difference between the instrumental running period.

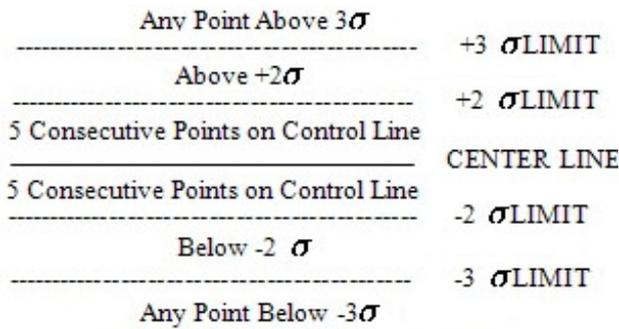


Fig. 5. An indicator chart of control chart plot with the main limits demonstrated

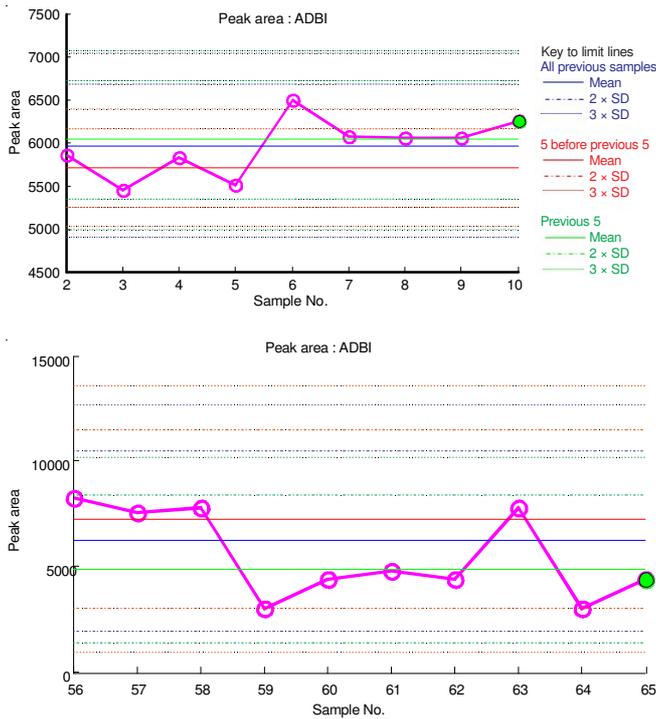


Fig. 6. Sample of Shewhart chart plot using first and last 10 reference samples

**Cumulative sum control chart:** The cumulative sum chart is commonly used in either tabular form or with the V-mask function to monitor the quality of data output from a procedure<sup>22</sup>. The cumulative sum chart can detect small changes (between 0-1 sigma), but for large shifts (1-2.5) the Shewhart type charts are equally effective and technically easier to use. The cumulative sum chart plots generate a visual representation of a gradual change from the mean. To generate cumulative sum charts in this study, the historic mean given by previous samples was used. Then, the cumulative distance from that mean was calculated for the most recent samples and cumulative test statistic on the right y-axial; see one example indicated in three green points with various previous data on the right of Fig. 7.

In contrast to the Shewhart chart, which plots a graph of the actual measurement (such as peak area, peak height, retention time, noise factor) at each point in time, the cumulative sum chart plots the cumulative difference of each reading from the mean. Therefore, if a value appears steady in the Shewhart chart, it will be different (and readily distinguishable) on the cumulative sum chart. Collecting  $M$  samples,

the cumulative sum chart can be plotted by the following equation:

$$S_M = \sum_{i=1}^M (x_i - \mu_0)$$

where,  $\mu_0$  is the estimate of the in-control mean,  $i = 1, 2, 3, \dots etc.$   $S_M$  is then plotted against the number of samples. Next, either the visual method or the tabular method can be used to generate the cumulative sum. The visual method, V-mask, is more appropriate for use to determine whether a process is out of control. In this study, we chose to use the tabular method. The parameters  $h$  and  $k$  were obtained by using the following equations:

$$h = 6 \cdot \sigma$$

$$k = 0.5 \times |a - b|$$

where  $a$  equals the mean of previous samples excluding the most recent 10 samples and  $b$  equals the mean of most recent 10 samples.  $\sigma$  is the known standard deviation of the sample means.  $h$  is highest limit and  $k$  is distance of the control limits.

The limit is then set using  $h$ , which in this study was set to  $6\sigma$  of the highest limit. Thus, the following quantities were calculated:

$$S_{h(i)} = \max(0, S_{h(i-1)} + x_i - \mu_0 - k)$$

$$S_{l(i)} = \max(0, S_{l(i-1)} + \mu_0 - k - x_i)$$

$$S_{\text{cusum-stat}(i)} = S_{h(i)} - S_{l(i)}$$

where,  $S_{h(0)}$  and  $S_{l(0)}$  are 0.  $S_h$  is higher cumulative sum tabular and  $S_l$  is lower cumulative sum tabular.  $\mu_0$  is estimate of the in-control mean and. When either  $S_{hi(i)}$  or  $S_{lo(i)}$  exceeds  $h$ , the process is considered to be out of control completely.

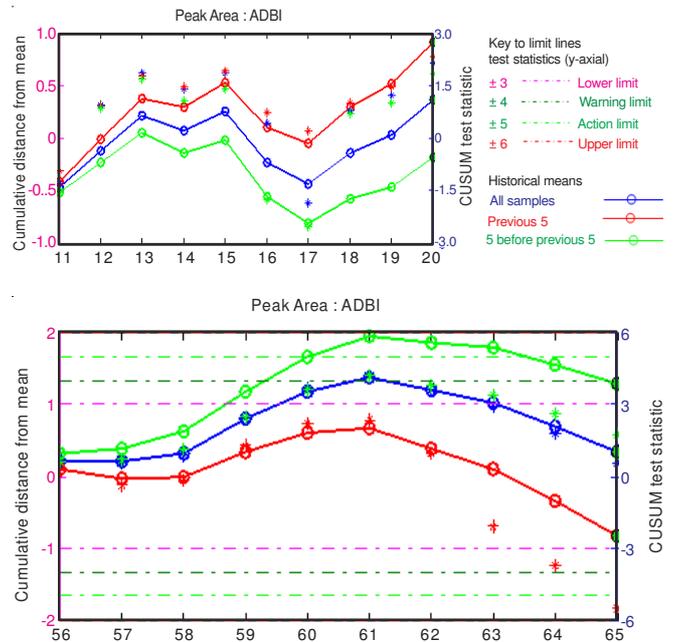


Fig. 7. An example of CUSUM chart plot using the first and last 20 reference samples

The three methods for calculating studied parameters in cumulative sum charts are the same as those used for the Shewhart charts. In Fig. 7, the cumulative distance from the

mean calculated for the latest 10 reference samples is shown by a red line, using the previous five samples as the historical mean; the green line indicates when the five samples before the previous five samples were used as the historical mean and the blue line indicates when the entire sample group was used to determine the historical mean. Here, the trend of the cumulative sum line is characterized by a dip down below the limits that occurred when the entire sample group was used to determine the historical mean. Thus, the noise factor level was concluded to have experienced some changes over the period of time encompassed by the continual procedure run on the GC-MS instrument (Fig.8). The cumulative sum value and the cumulative sum test statistic are plotted on the same graph, but using different vertical axes (left side and right side, respectively).

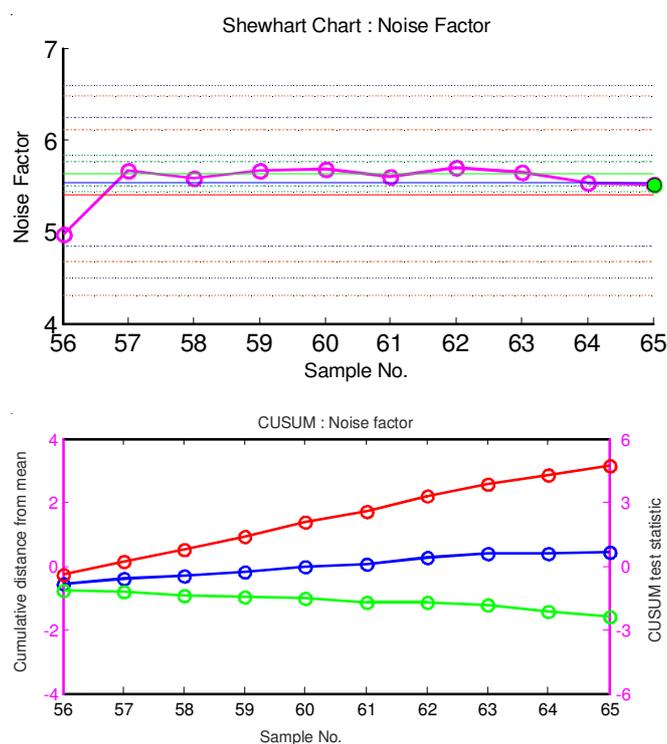


Fig. 8. Average value of noise factor (equals to 5.58). The Shewhart and CUSUM charts of last 10 samples

As stated above, the cumulative sum chart is capable of revealing an individual change in a particular parameter that is undetectable by the Shewhart chart. This is illustrated in Fig. 7, where the blue lines crossing the pink lines are slightly different for the two vertical axes. The pink lines show the cumulative sum (and are read using the pink vertical axis on the left), whereas the blue lines crossing over show the cumulative sum test statistic and indicate how the cumulative deviation from the mean compares to the mean itself (and are read using the blue y-axis on the right). The test statistic value was set to 5 for the warning limit and 6 for the action limit.

#### ANOVA statistics applied to the quality control samples:

A key aim of quality control experimentation is to see how significant the contribution of a particular factor is to the outcome data. We performed statistical analysis using the peak heights, retention time and peak areas from the quality control samples to determine which factors may have impacted

(biased) the quality of outcome data measured. Degrees of freedom for the experiments were 65 and degrees of freedom for the parameters were 3. Three factors were used in this analysis: experimental conditions (with/without agitation); maintained system and column.

ANOVA was performed to determine whether a factor had a significant influence on the response and what factors, if any, were more significant than others<sup>23</sup>. A linear model was formed as follows:

$$y = D \cdot b$$

$$b = (y^T \cdot y)^{-1} \cdot y \cdot D$$

where  $y$  is a vector of responses (in this example,  $y$  corresponded to the peak height of the internal standard for 35 reference samples),  $D$  is a coded design matrix and  $b$  is the coefficient for the terms in the equation that relate the response to the value of each factor. The greater the value of these coefficients the more significant the factor is considered to be, provided that each variable is coded in the same way. Once the coefficients were calculated, then a new predicted was able to be calculated by:

$$\hat{y} = D \cdot b$$

In present analysis, three factors were studied, including w/o agitation, maintained systems and columns. A linear relationship of the form was determined by:

$$y \approx b_0 + \sum_{k=1}^K b_k x_k$$

where, there were  $k = 1, 2, 3$  in this case.

The sum of squares residual error for each peak height ( $i$ ) was then calculated by:

$$S_{\text{rep}} = \sum_{i=1}^M (y_i - \bar{y}_i)^2$$

The total sum of squares for the raw peak heights was determined by:

$$S_{\text{total}} = \sum_{i=1}^M y_i^2$$

The sum of squares of the replicated differences was calculated by:

$$S_{\text{resid}} = \sum_{i=1}^M (y_i - \hat{y}_i)^2$$

where,  $\bar{y}_i$  is the mean response at each unique factor. The sum of squares for the predicted data was then given by the following:

$$S_{\text{reg}} = \sum_{i=1}^M \hat{y}_i^2$$

The lack-of-fit sum of square error was calculated by:

$$S_{\text{lof}} = \sum_{i=1}^M (\bar{y}_i - \hat{y}_i)^2 = S_{\text{resid}} - S_{\text{rep}}$$

The ratio of F was determined by:

$$F_{\text{ratio}} = \frac{S_{\text{lof}}}{S_{\text{rep}}}$$

The  $F_{\text{stat}}$  was calculated as follows:

$$F_{\text{stat}} = \frac{S_{\text{lof}} / (M - K - R)}{S_{\text{rep}} / R}$$

TABLE-3  
RELATIONSHIP BETWEEN THE PARAMETERS IN THE CUSUM CHART

Sample	1	2	3	4	5	6	7	8	9	10
ADBI	10.07	10.06	10.06	10.06	10.06	10.06	10.06	10.06	10.06	10.06
AHMI	10.76	10.75	10.76	10.75	10.75	10.75	10.75	10.75	10.75	10.75
MA	12.11	12.11	12.11	12.11	12.11	12.11	12.11	12.11	12.11	12.11
HHCB	12.53	12.53	12.53	12.53	12.53	12.53	12.53	12.53	12.53	12.53
AHTN	12.72	12.72	12.72	12.72	12.68	12.72	12.68	12.68	12.68	12.68
MX	12.64	12.64	12.64	12.68	12.64	12.64	12.64	12.64	12.64	12.64
MK	15.41	15.40	15.40	15.41	15.38	15.40	15.38	15.38	15.38	15.38
MM	13.22	13.22	13.22	13.22	13.22	13.22	13.18	13.18	13.18	13.18
MT	14.35	14.33	14.32	14.33	14.33	14.33	14.32	14.33	14.32	14.33
HHCB-lactone	21.23	21.23	21.23	21.23	21.22	21.22	21.20	21.20	21.19	21.20
d15_MX	12.38	12.38	12.34	12.38	12.38	12.34	12.34	12.34	12.34	12.34
OTNE	9.47	9.47	9.47	9.47	9.47	9.47	9.46	9.46	9.46	9.46
D10-phe	11.75	11.75	11.75	11.75	11.75	11.75	11.75	11.75	11.75	11.75
mean	12.97	12.96	12.96	12.97	12.96	12.96	12.95	12.95	12.95	12.95
x2	3.80	7.79	11.70	18.37	22.86	28.46	40.96	44.61	45.35	48.37
x3	12.53	12.72	12.64	15.40	13.22	14.33	21.23	12.38	9.47	11.75
Cp	3.30	6.79	10.20	16.37	20.36	25.46	37.46	40.61	40.85	43.37

where, M is the number of samples, K is the number of factors and R is the number of replicated samples. A total of 65 quality control samples were tested for confounding factors. The experimental details of the samples were recorded and employed in the analysis.

## RESULTS AND DISCUSSION

Quality control charts, including the Shewhart and cumulative sum charts, are used to monitor the progress of instrumental analysis and manage routine procedures to ensure high quality data output. Quality control is carried out with the aim of avoiding systemic error and generating measurements that are as accurate and reliable as possible. In this study, two well-established types of control charts were evaluated for their combined ability to analyze the quality of GC and MS instruments producing continuous data on objective compounds.

Control charts are also considered basic and powerful tools for statistical process control (SPC). There are three approaches by which control charts determine quality of data output. In the first all historical data are analyzed in order to decide if the overall process is within statistical control and to estimate the in-control parameters of the process. It is important that all special causes are detected in this particular situation because this leads to a better understanding of the process. In the second approach, the previous five samples are considered as representatives of the historical data and applied to the analysis to decide if the process is in statistical control of the most recent samples. In the third approach, the historical data is determined from the five samples before the previous five samples. A cumulative sum type chart is most appropriate for the detection of linear trends in individual observations<sup>24</sup>, especially as compared to the Shewhart chart. The relationship between the parameters in cumulative sum can reveal relatively minor errors (and out-of-control conditions) in the ongoing data process (Table-3). The  $\bar{x}$  control charts themselves are composed of an x-axis (number of reference samples), y-axis (characterized values of control chart) and a central line (the mean of the experimental results); within the chart, varied

standardized deviations or cumulative statistical values represent the thresholds for warning limits and action limits. On the other hand, the Shewhart chart (also known as a 'process-behaviour' chart) is most appropriate for determining whether a manufacturing or business process is in a state of statistical control or not. If the chart indicates that the process is currently under control, then it can be used with confidence to predict the future performance of the process. If the chart indicates that the process being monitored is not in control, the pattern it reveals can help determine the source of variation that needs to be eliminated in order for the process to be brought back into control. When a value falls outside of the defined limits of the control chart, the points responsible for the underlying process are expected to determine whether a special cause has occurred. If one has, then that cause should be targeted for elimination, if possible. Since the control limits are evaluated each time that a point is added to the chart, it readily follows that every control chart will eventually signal the possible presence of a special cause, even though one may not have actually occurred.

In present study, a total of 65 quality control samples were investigated to identify the presence of specific confounding factors. For the response, we used the peak heights of the internal standards, which are very large. The F-ratio was used to show how significant a single variable (or mean sum of squares) was relative to another variable. The F-stat was used to describe the various probability levels and the F-stat levels of these three factors (w/o agitation, maintained systems and columns) were determined to be much closer (Table-4). F-probability level of these three factors are much closer. These three factors in the ANOVA tables revealed that all were not important factors for the variance of measurements of the consistent peaks in the reference samples after comparing their F-stats that were much close (F-probability < 1).

## Conclusion

By performing continuous monitoring with Shewhart and cumulative sum charts, minor errors in continuous GC and MS instrument analysis of a large sample group were able to be detected in real-time. The chromatograms of the reference

TABLE-4  
RESULTS OF ANOVA DEMONSTRATED THE AVERAGE PARAMETERS UNDER VARIOUS INSTRUMENTAL CONDITIONS. THE REFERENCE SAMPLES SHOWED F-PROBABILITY CLOSE TO 1, INDICATING THAT THE DIFFERENCE WOULD NOT BE SPECIFIC IN THE MONITORING SYSTEM

(A) ANOVA RESULT OF AVERAGE PEAK HEIGHTS OF THE REFERENCE SAMPLES						
ANOVA results of average peak heights						
No.	Factor	S. reg.	S. resid.	S. lof.	F-stat	F-probability
1	W/o agitation	235.41	185.63	163.74	7.48	0.89
2	System	197.07	223.97	202.08	9.23	0.91
3	Column	185.00	236.04	214.15	9.78	0.91
(B) ANOVA RESULT OF AVERAGE RETENTION TIMES OF THE REFERENCE SAMPLES						
ANOVA results of average retention times						
No.	Factor	S. reg.	S. resid.	S. lof.	F-stat	F-probability
1	W/o agitation	86.10	26.51	17.54	1.96	0.70
2	System	79.66	32.95	23.98	2.67	0.76
3	Column	74.76	37.85	28.88	3.22	0.79
(C) ANOVA RESULT OF AVERAGE PEAK AREAS OF THE REFERENCE SAMPLES, WHICH WERE SCALED BY 10 <sup>6</sup>						
ANOVA results of average peak areas						
No.	Factor	S. reg.	S. resid.	S. lof.	F-stat	F-probability
1	W/o agitation	7.73	4.81	4.09	5.62	0.86
2	System	6.67	5.87	5.15	7.08	0.88
3	Column	7.66	4.88	4.16	5.72	0.86

samples revealed that the spectra of the peaks were very stable over a long period of time.

The Shewhart control chart used here was characterized by 3-sigma limits; this false alarm can occur on average varied observation and should be kept in mind when using this type of chart. Meanwhile, if a special cause actually does occur, it may not be of sufficient magnitude for the chart to produce an immediate alarm condition. If a special cause is suspected, it can be verified by measuring the change in the mean and/or variance of the process in question. When those changes are quantified, it is possible to determine the out-of-control limit for the chart. It turns out that Shewhart charts are effective at detecting large changes in the process mean or variance, as their out-of-control limits are fairly short in these cases. Cumulative sum charts, on the other hand, plot the cumulative sum of the deviations between each data point (a sample average) and a reference value. Therefore, an operator studying a cumulative sum chart should be concerned with the slope of the plotted line, not just the distance between plotted points and the center line. ANOVA results provided that these factors had no significant effects on the measurements of the consistent signals. A further complication that must be kept in mind is the differences that can arise as a result of the length of the column used in the GC-MS instrument; for example, the volatile profile may change with time as the concentration of more volatile compounds changes during the run process.

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