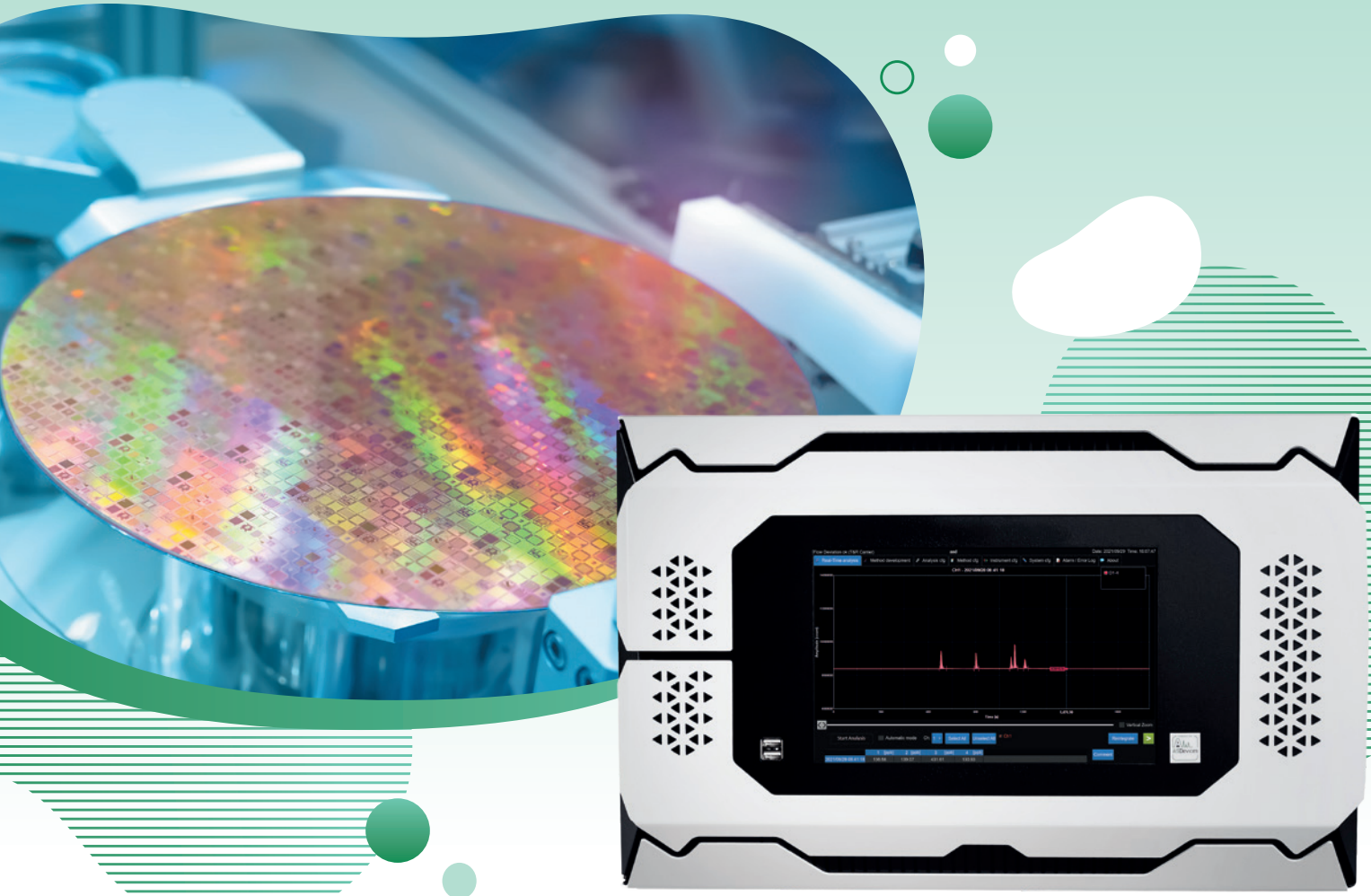

APPLICATION NOTE 20

UNDERSTANDING LIMIT OF DETECTION VALIDATION AT ULTRA-TRACE LEVEL

Not all limit of detection are the same

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ASDevices

Introduction

We have been involved in gas chromatography for over 3 decades. We have invented many technologies to advance this field and improve the reliability and performance of chromatograph. Not only new application development, but new ways of doing things. Most of our work has been in ultra trace analysis, in the ppt and ppb level. At those levels, you are always pushing the limits to determine the ultimate question: What limit of detection (LOD) can you achieve? Our question has always been, do you want a marketing LOD or a scientific one?, because there are many ways of calculating it. So, when looking at a LOD, it is important to understand the way it was calculated to do a like for like comparison.

Limit of Detection (LOD)

LOD can be calculated in many ways. There are many definitions. We are presenting two LOD methods in detection limit (MDL). Figure 1 is a representation of what is taken into account by each of the methods we are presenting.

A very common one is purely based on signal to noise (SNR) calculation. This is the weakest approximation of "method LOD". This method is often referred to as "minimum detectability" (MD) [1] because it provides the lowest LOD. The formula is: where k is a multiplier, typically 3, C is the concentration, S is the peak height and N is the baseline peak to peak noise over the length of peak width. Basically, the LOD is when the analyte signal is 3 time the height of the noise.

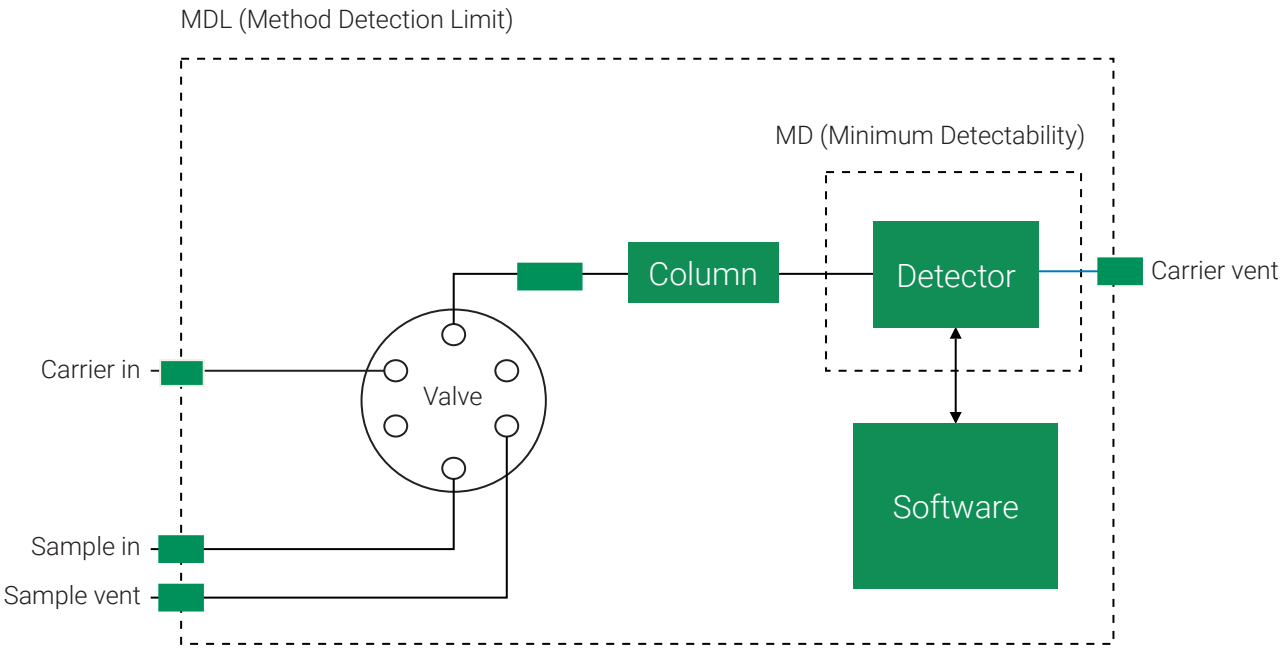
Marketers [3, 5] like this method for obvious reasons. However, it leaves out many of the methodology and system errors. As such, this method is good to compare the performance among similar detectors, but not robust enough to properly characterize the "method LOD". In chromatography, the detector sensitivity is only one factor that contributes to the method performance. Valve performance, baseline drift, chromatographic columns, signal post processing algorithms are only some of the factors that contributes to the overall performance. Therefore, we never rely on this method to specify the performance of our instruments. We only use this method to evaluate the performance of detectors for a specific gas molecule [4].

At ultra-trace level, it is even more important to use a proper method, because at very low concentrations, ppt and ppb, it is possible to lose the impurities through various processes like surface absorption, diffusion, and mixing. Even with a MD of let's say 100 ppt, it is possible that no signal will be detected at 1000 ppt because it is absorbed by the chromatographic hardware.

In that case, a LOD of 100 ppt would have been a false statement. It is not uncommon for some analytical instrument to have such a dead band where there are no signal variations. The LOD must consequently be above that level.

Before we explain the method detection (MDL), which is the one we use, it is important to explain further why MD method is not a robust one.

Figure 1 – LOD: MD vs MDL



System contribution to peak broadening

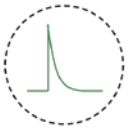
Chromatographic systems are made of multiple components: valves, fittings, tubes, detectors, etc.. Each of these components have an impact on the system performance.

Too many chromatographers assume that some parts, like fittings are almost perfect. Experienced chromatographers know very well that fittings are more than just a connection. They have dead volumes which influence peak shape and may even cause sample dilution.

When a LOD is purely characterized based on signal to noise, an important assumption is made. The peak shape and influences from various chromatographic components remain constant regardless of concentration. This is a false assumption, especially with complex chromatographic systems as the overall performance is impacted by the summation of all components. Moreover, at trace level, those phenomena are more pronounced.

In 1966, the book "Advances in Chromatography" [2] was published and contains a complete chapter, "Extracolumn Contribution to Chromatographic Band Broadening", on that very subject. This book, which is a mathematical analysis of real phenomenon, analyzes the impacts of each chromatographic components on the peak shape and consequently system performance. The purpose of this section is not to explain all the details but create an awareness. The author describes, using mathematical transfer functions, what is the contribution for each of them. The most common ones are represented in table 1.

Table 1 – Chromatographic components transfer function

Transfer function	Normalized function	Where found in GC system
	$\left(\frac{1}{\sigma\sqrt{2\pi}}\right)e^{-\left(\frac{(t-tr)^2}{2\sigma^2}\right)}$	Chromatographic column Syringe injection
	$\frac{1}{\tau}$	Gas injection valve
	$\left(\frac{1}{\tau}\right)e^{\left(\frac{-t}{\tau}\right)}$	Fittings Slow vaporisation chamber

What is a transfer function?

In simplistic terms and as a minimum, a system is made of an input, a transfer function, and an output (figure 2). A transfer function is a mathematical function that transform the input into the output. For chromatographers, the easiest one to understand is a chromatographic column (figure 3). Let's take a sample gas containing only one impurity. If we were to measure the peak shape that is coming out of the valve, the peak shape would be most likely square. However, if the same impurity is injected through a chromatographic column, the peak shape which has a square shape at the input, is going to be transformed into a gaussian shape. This is the impact of the column gaussian transfer function. So, a transfer function can be seen as a process that changes an input signal into an output signal.

Figure 2 – Transfer function

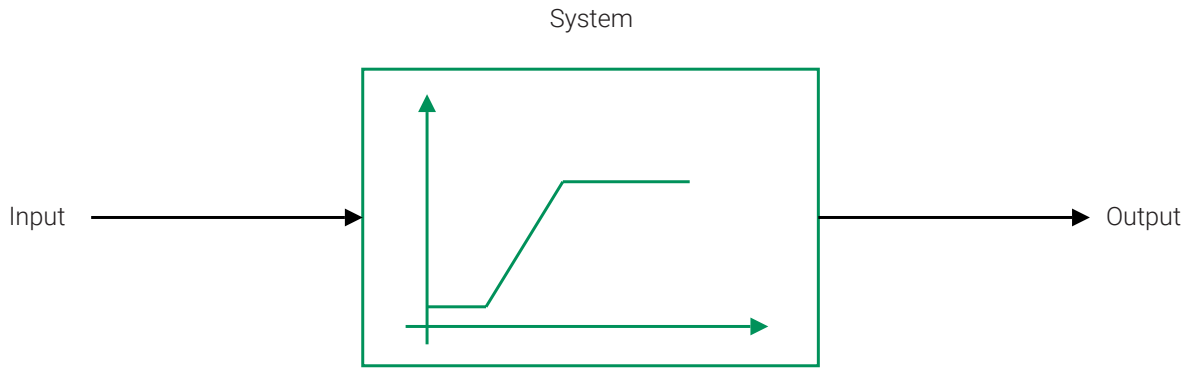
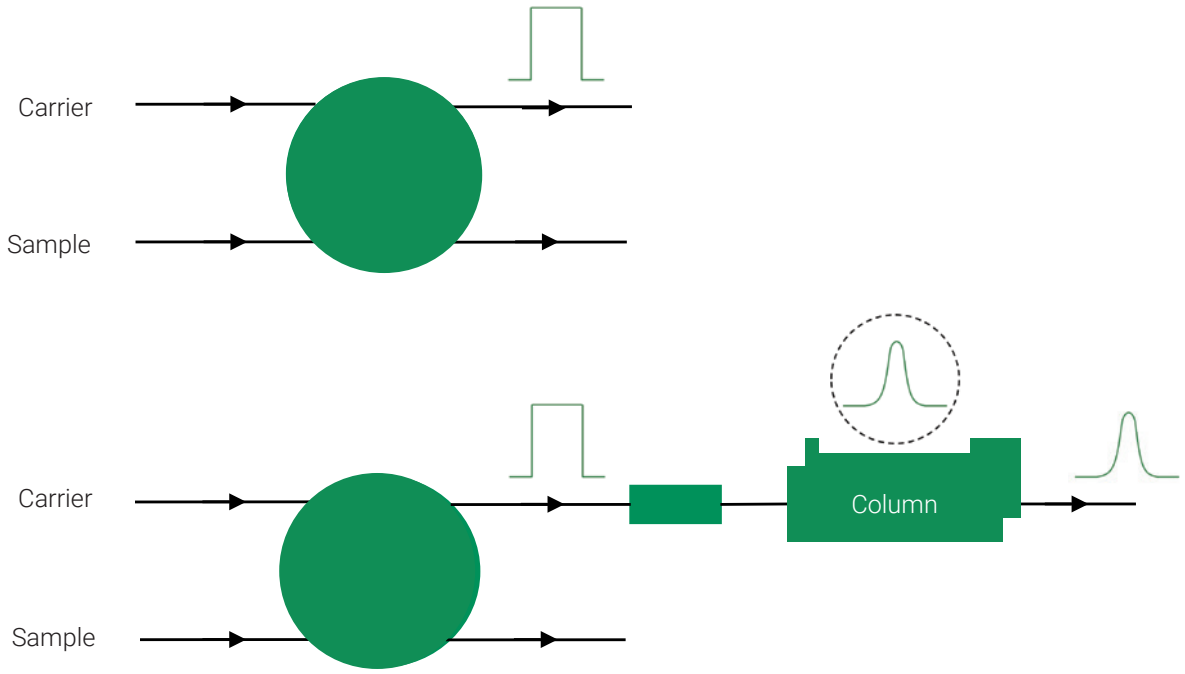


Figure 3 – Impact of components transfer functions



Fitting – More complex than one might think

Let's take a simple fitting. A fitting is simply a connection that can be used to connect a valve to a chromatographic column. As most fittings are imperfect (figure 4a), two phenomena occur: Diffusion and Mixing. Figure 5a represents the impact of those two phenomena on gas molecules that are flowing through it. The diffusion principle causes some broadening. When measuring percentage levels, this impact may not be so important. However, when measuring ppt levels, this volume that may appear small, is in fact a huge volume for a small quantity of molecules to diffuse into. It will consequently negatively impact the peak shape at trace level and even more when near the instrument LOD.

The mixing, as its name implies, is causing a dilution. As the molecules diffuse into the dead volume, the mixing causes some dilution. Again, it may be a small dilution, but at trace level, not something to ignore.

As such, it is important to use appropriate high quality analytical fittings. ASDevices LipLOK™ is a very good example. Such fittings have been designed and optimized to remove dead volume and avoid such issues (figures 4b and 5b).

Figure 4a – Standard fitting dead volume

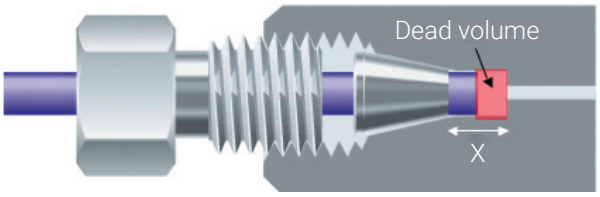
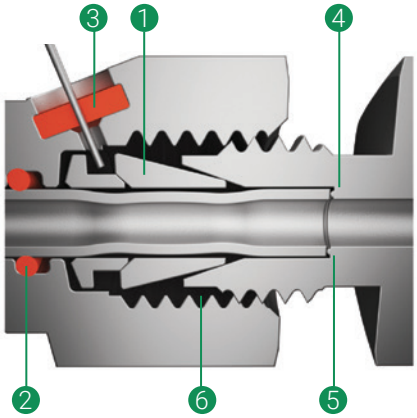


Figure 4b – LipLOK™ fitting, no dead volume



1) Standard front ferrules: Second level of sealing and tubing swaging action that prevent tube expulsion under high pressure/vibration environment

2) Tubing surface seal and nut sealing ring: Provide concentration chamber sealing

3) Septum: Leak detection sniffing with syringe

4) Coated sealing ring: First level of sealing

5) No dead volume: Direct flow through design

6) Leak concentration chamber: Sniffing – detect the smallest leaks by accumulating and concentrating them. Tracer – pressurize the chamber with a tracer gas for leak integrity test

Figure 5a – Type of peak spreading in standard fittings due to diameter change

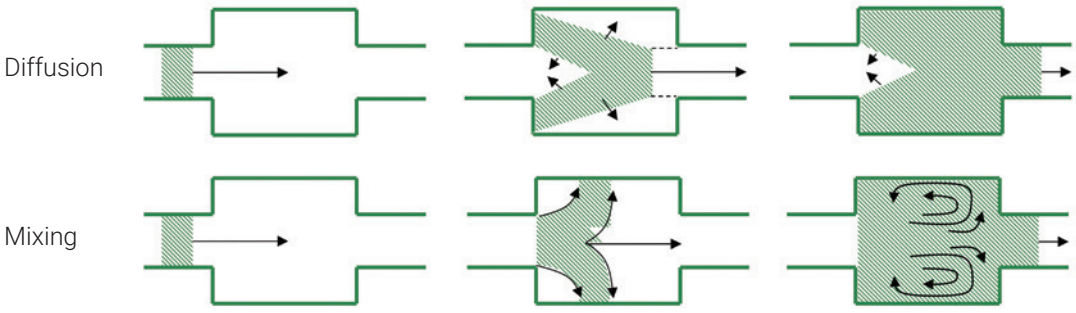
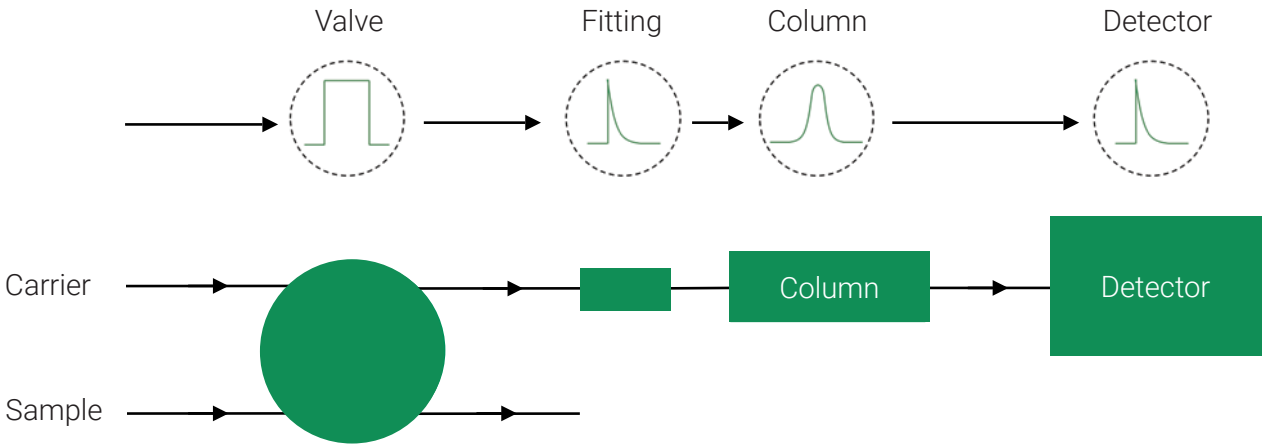


Figure 5b – No spreading with ASDevices LipLOK™ fitting

Similar phenomenon occurs in valves, detectors, columns, etc. Figure 6 is a representation of a simple chromatographic method. It can be understood that all those factors will negatively impact the measurement and a strong argument against only using SNR calculation. For that very reason, a robust LOD method most take all of these into account.

Figure 6 – Typical transfer function in a simple GC method



Finally - Generating reliable ultra-trace reference gases

Before we even talk about LOD calculation, we need to address a very important question: how can we generate a reliable ultra-trace reference gas in the ppb or even ppt level?

Ultra-trace gas analysis is a field we know very well and we have developed expertise to generate trace level references. During that period, we have continuously innovated to improve the reliability of gas chromatographs. Properly testing them is part of the equation. We needed a dilution system capable of generating high dilution ratios while preserving sample integrity to generate ppt and ppb references from ppm level gas cylinders. Moreover, we needed a dilution system that was not impacting the gas composition of the sample to generate ultra-trace sulfur references or handle any other type of reactive molecules. We found no reliable solution on the market that was meeting our expectations. For that very reason, we have developed and commercialized a high-quality dilution system, the GCS, for ultra-trace analytical system validation. This system quickly became a reference in the market.

The GCS is based on our proprietary electronics pressure controller (EPC) that is purged to be leak tight and temperature compensated for precision. The highly precise and stable pressure is used to generate a gas flow through highly precise laser calibrated orifice that are operated in sonic flow regime. The sophisticated mathematical model is used to precisely generate flows and consequently generate precise dilutions. The orifices are used in the sonic regime where the flow is only a function of the inlet pressure. This consideration is important to avoid any impact on the dilution ratio from the outlet pressure which could fluctuate due to the analytical instrument.

Figure 7 – GCS dilution system

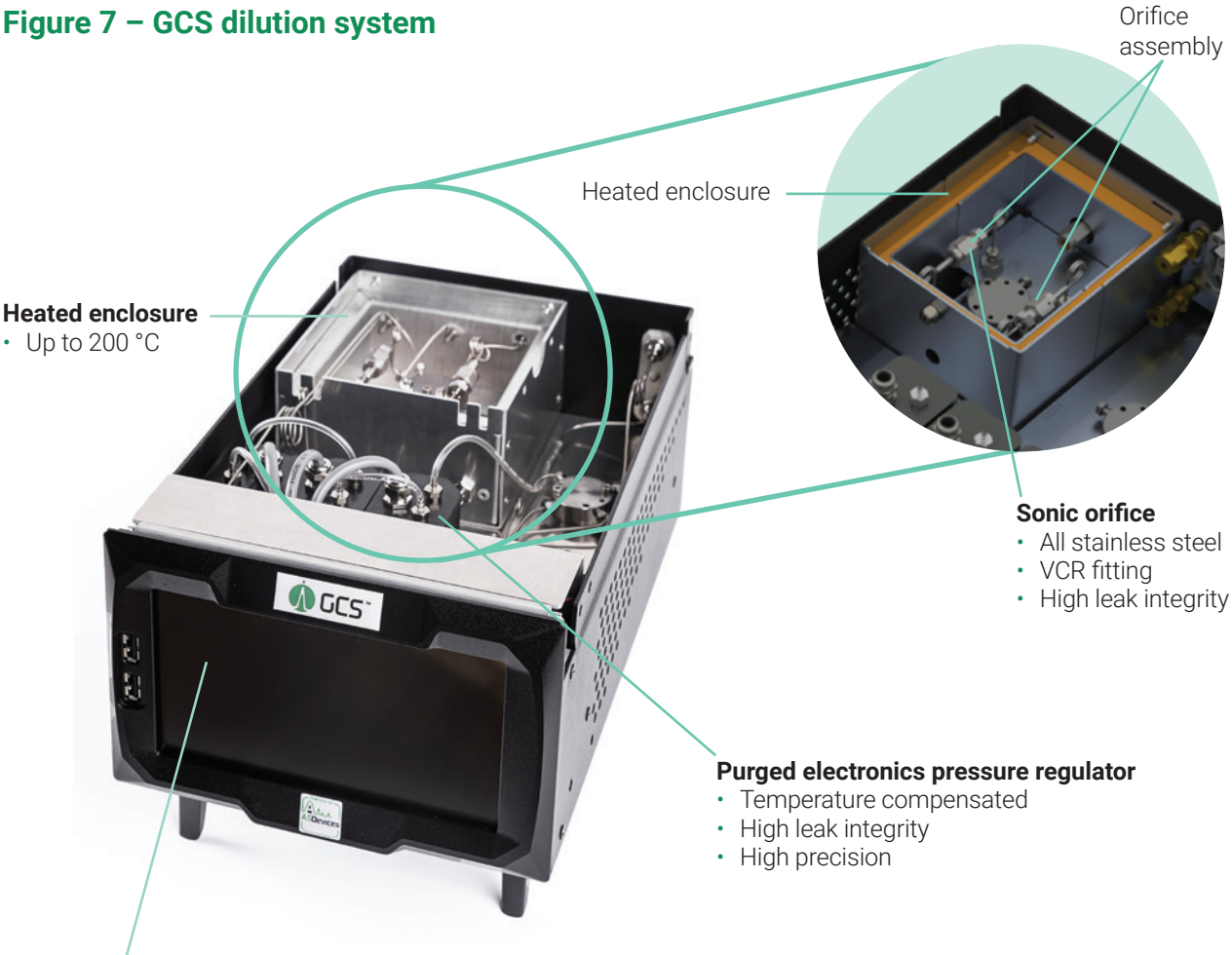
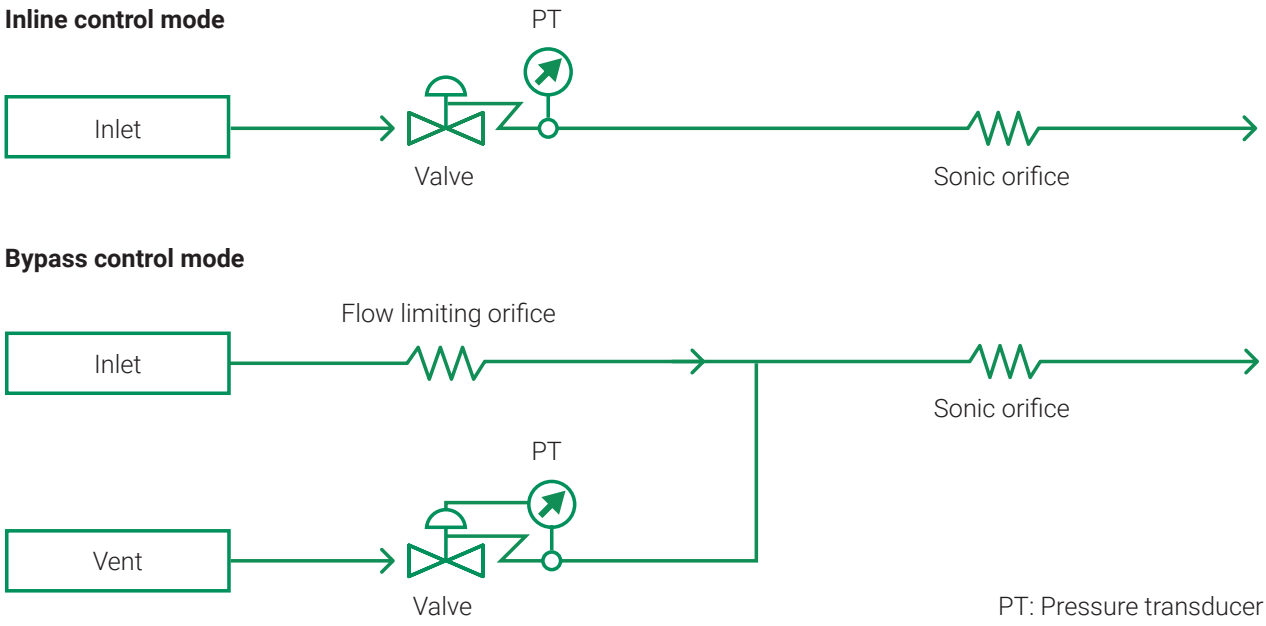


Figure 8 – GCS dilution system, inline vs bypass pressure control



If this was not enough, we offer a version that controls the pressure in bypass. In this configuration, the EPC is not inline with the sample gas, but installed in a bypass vent. This configuration makes sure that no control elements, such as valve and pressure sensor, are in contact with the gas. This way, the sample content is not compromised, and this is very important. Finally, we control the temperature for stability and avoid sample condensation. With such a system, we can achieve dilution ratio of up to 1:10000 with a precision of 0.5%.

Method Detection Limit (MDL) – A robust LOD method

Because of all the phenomenon we previously described, we use a much more robust method when we calculate our LOD, because we want a LOD that is true in the real world and considers the entire analytical system. We want a LOD that can be achieved by the analytical method, not only the detector.

To calculate our LOD, we use a standardized method which as been defined by the International Union of Pure and Applied Chemistry (IUPAC) [1]. The obtained LOD is called Method Detection Limit (MDL). This method is a robust statistical approach. It incorporates the variability in the blanks or to be even more robust, a reference gas having impurity levels near the expected LOD. Generating such an ultra-trace reference gas is not something trivial and a reason why many GC manufacturers avoid this method and prefer the one based on SNR.

Instead of calculating the SNR based on a reference impurity value, this method requires 10 or more consecutive analysis. Less can be used, but a multiplier is necessary to make sure 99.67% confidence level (see table 2) is preserved. This method is more robust, because it is based on results calculated by the instrument and influenced by all factors. With the advance in signal processing/filtering, this method also makes sure that software enhanced signals are taken into account in the calculation. For our technology, it makes sure that post signal processing such as our advanced signal processing algorithm enhanced limit of detection (eLOD) is for example part of the equation. It also does not involve human decisions which can be different from one person to the other. This is purely a statistical approached based a well understood and proven mathematics.

The MDL formula is calculated using the following formula:

$$MDL = k\sigma$$

Where k is a factor from table 2 and σ is the standard deviation of the calculated results from the instrument.

Table 2 – MDL factor

Number of analysis	K factor for 99.67% confidence
5	5.507
10	3.957
15	3.586
20	3.422
25	3.330
30	3.270

The IUPAC method will always provide a higher LOD when compared to the method based on SNR. It is however much more representative. As most companies still use MD method, we also publish the performance based on that method so that apples can be compared with apples.

> ***Note:*** *For the MDL method, it is important to use a reference gas that is as close as possible to the expected limit of detection. Otherwise, the system performance will be underestimated.*

Other GC manufacturers uses this definition but are reducing the confidence level to 95% [6]. Also, they use a one-sided statistical test instead of a two-sided one. This is helpful to publish lower limits of detections, but this is not the standard definition and not approved by the analytical community. Also, they perform the test using a pure gas (no impurities). This can be misleading as mentioned previously. In trace analysis, there is sometimes a deadband where no measurement occurs.

Therefore, we use our GCS dilution system to fully validate all the chromatographic methods we develop.

As analytical instrument manufacturers This is the very reason of this document. Not all LODs are the same. This document educates our customer on that important topic.

A real life example

Here is an example based on a ASDevices KA8000Plus chromatograph configured to measure permanent gases at ultra-trace level within a 0-1000 ppb range. This is a very difficult measurement, one for which we are expert. As this application is related to the semiconductor industry which needs to properly understand the quality of the gas they are using, they really care about LOD and this has been a major topic for us for many decades.

In this example, we measure the LOD using both methods. To do so, we have generated a 4 ppb reference gas using our GCS calibration system followed by a 700 ppt one.

At first, we run 10 consecutive analyses with the 4 ppb reference gas. The data is used to calculate the LOD based on the two previously discussed method. In addition to those 10 analyses, we also captured the noise which will be used to calculate the SNR for the MD method.

Table 3 represents the calculated values from our KA8000 instruments. Figure 9 is a representation of the 4 ppb peaks and the baseline noise.

Table 3 – MDL calculation

	Measurement (ppb)				
Analysis #	**N_2**	**H_2**	**CH_4**	**CO**	**CO_2**
1	3.59	3.79	3.47	4.26	3.99
2	3.93	3.96	3.46	4.68	3.7
3	3.96	3.91	3.59	4.54	3.61
4	3.97	3.94	3.61	4.58	3.87
5	3.83	3.85	3.66	4.61	3.73
6	3.79	3.89	3.67	4.5	3.86
7	3.47	3.77	3.53	4.5	3.54
8	3.77	3.89	3.50	4.65	3.71
9	3.77	3.87	3.48	4.55	3.73
10	3.95	3.92	3.52	4.34	3.98
Average (ppb)	3.803	3.879	3.549	4.521	3.772
σ (ppb)	0.167	0.061	0.078	0.132	0.149
MDL (ppb)	0.658	0.242	0.308	0.521	0.590

Figure 9 – SNR calculation example

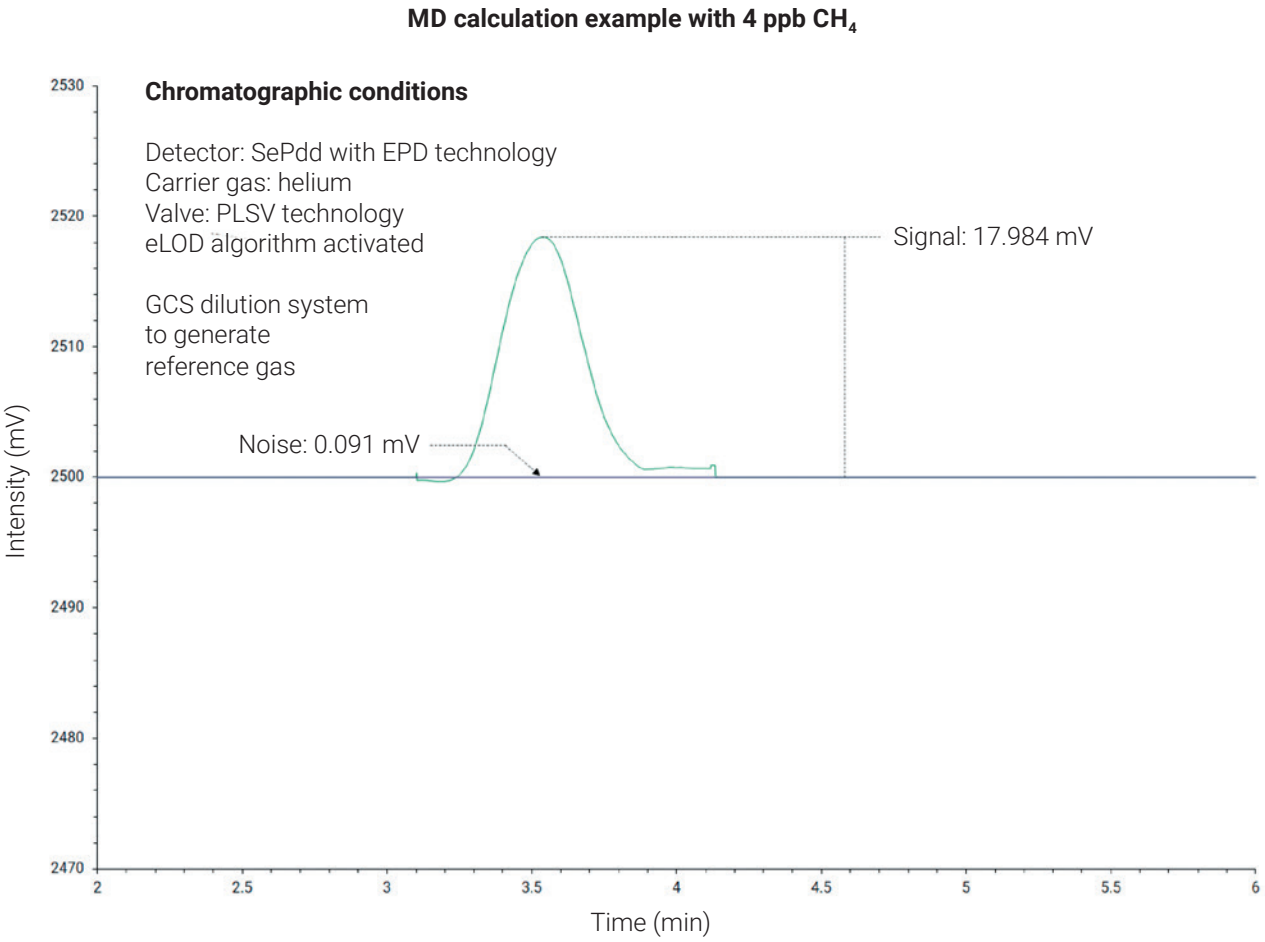


Table 4 demonstrates the different results obtained using both methods. There is almost a factor 10 between both methods. So not all LODs are equal and understanding how they are calculated is of outmost importance.

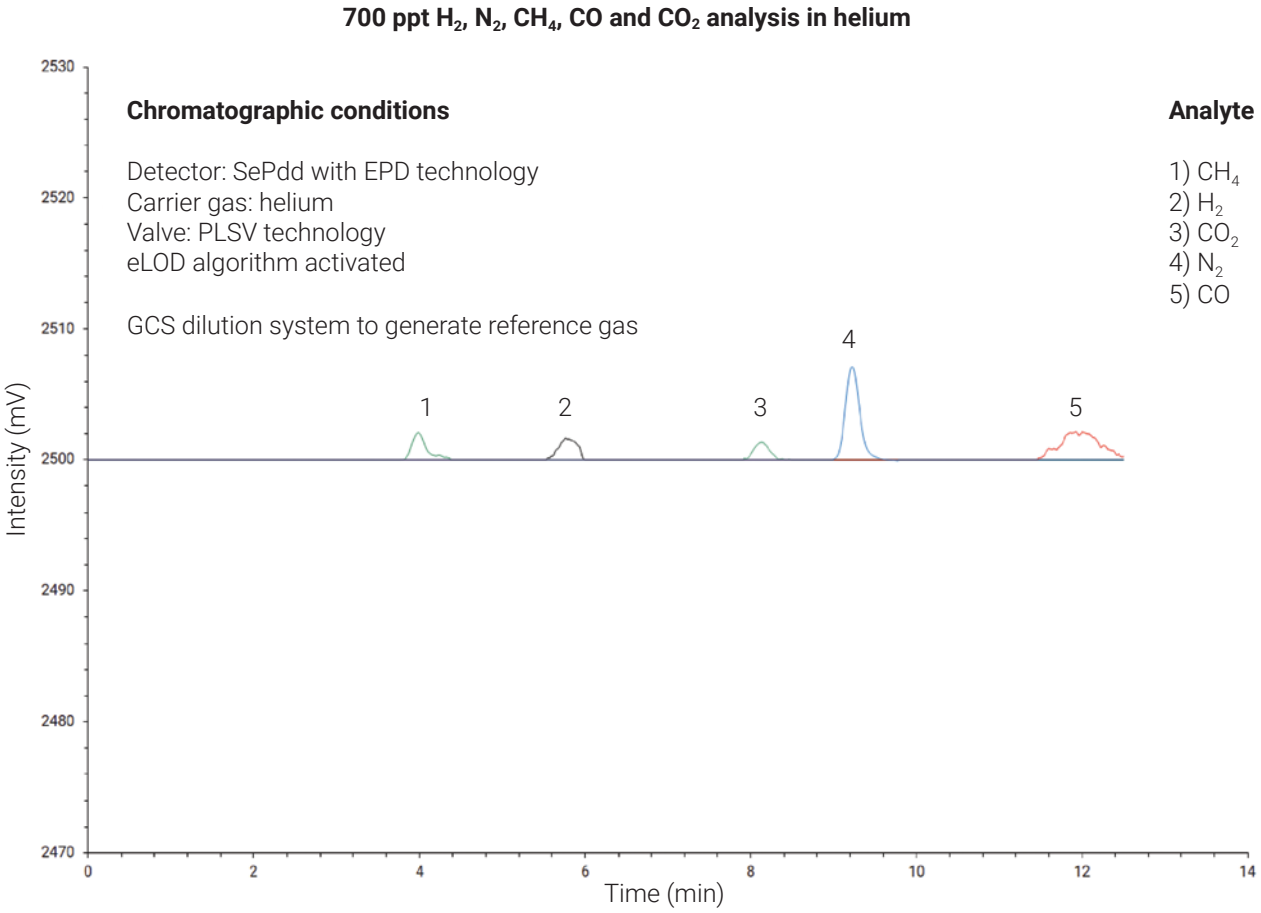
Table 4 – LOD comparison

LOD	N_2	H_2	CH_4	CO	CO_2
MD (ppt)	54.1	50.1	61.5	72.1	69.1
MDL (ppt)	242.0	658.0	308.0	521.0	590.0

Pushing the limits

To further demonstrate the capability of our technologies, we generated a 700 ppt reference gas as we wanted the reference gas to be closer to what we believe is the true performance. Generating such a reference in a stable manner requires skills, time and knowhow. At those levels, the sampling system quality and associated hardware must be of extremely high quality. Otherwise, you may simply lose the analytes or have to wait for days to let the system stabilize. As we have been in this field for over 30 years, we understand very well what is required. Figure 10 is the chromatogram we achieved at 700 ppt. We can clearly see that we still have nice chromatographic peaks that are easily quantifiable. This level is well above the LOD of detection.

Figure 10 – 700 ppt chromatogram



This chromatogram shows that the value obtained in table 4 are over the true performance of this instrument and this is due to the fact that the data in table 4 were obtained with a 4 ppb (4000 ppt) reference instead of 700 ppt. As the MDL method takes into account the system repeatability, a higher reference value causes an under estimation of the true performance. For many, generating reference gases below 1 ppb is simply impossible. Not for us. Based on the 700 ppt test, the calculated performance is shown in table 5. We can see that the MD results remain the same but that the MDL values are now getting closer to the MD ones. The MDL method still remains a more robust method as is takes into accounts the entire system and not simply the detector.

Table 5 – LOD comparison

LOD	N_2	H_2	CH_4	CO	CO_2
MD (ppt)	54.1	50.1	61.5	72.1	69.1
MDL (ppt)	62.0	97.0	92.0	188.0	94.0

Conclusion

In conclusion, unless you know the definition used to calculate the limit of detection of your instrument, it is difficult to compare the data from different instruments suppliers. As the aim of a process chromatograph is to measure analytes that are coming from a process, we use a definition that represents the true performance of the instrument. The minimum detectability definition is simple, but not robust enough for a process chromatograph that involves many components such as valves, fittings, columns, detectors and software. The method detection limit is well documented and proven to be a robust method and this is what we use. The challenge with such a method is generating a reliable trace reference. This is why many process chromatograph suppliers avoid it, but not us. We have a principle; we have to be able to demonstrate what we claim.

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