Questions of Quality

(Nearly) All You Wanted To Know About A/D Converters...

We all have them in our laboratories, but usually never worry about them. They can be hidden inside a chromatograph, pH meter, spectrophotometer or simply placed on a bench. What are they? Analogue-to-digital (A/D) converters, of course! Just read the display or connect the detector leads to the back of the A/D box, set up the method and away you go. Collect the data, interpret the chromatogram and print out the results. Easy really — just like falling off a log. No problems! Or are there?

Chris Burgess,
Burgess Consultancy, Darlington, County Durham, UK,
Dianna G. Jones,
SciOptics Corp., Orlando, Florida, USA,
and R.D. McDowall,
McDowall Consulting, Bromley, Kent, UK.

Important as these devices are in other instruments, we want to focus on the analogue-to-digital (A/D) converters in chromatography data systems. These vital components are used throughout our laboratories in stand-alone chromatographs, integrators, personal computer data systems and networked data systems. Analogue-to-digital converters are either integrated in systems or are discrete units that stand alone on our laboratory benches.

With the emphasis on equipment qualification, as a result of the Barr Ruling (1, 2), it is important that chromatographers can understand their data systems and can demonstrate their suitability for use.

Analogue-to-digital units are universally used, but never universally understood. Do you walk around with a paper bag on your head? Of course not, but you might as well do if you don't know how your equipment works. Therefore, as the A/D chips are at the heart of a chromatography data system, we need to ask some questions:

- What are A/D converters and how do they work?
- How are A/D units in chromatography data systems being used?
- What are the realistic approaches for user testing?
- What is the impact on operational and performance qualification (OQ and PQ) for validation?

Analogue Data

Before answering these questions we need to get back to the Jurassic Age of chromatography. Yes, it's pen-and-paper time, or, to be more specific, chart-recorder time. The advantages of a chart recorder for a chromatogram are:

- a continuous real-time data plot
- visual inspection to see any over- and under-range peaks.

Remember that the chart recorder itself contains signal processing elements, such as response time circuitry, which smooth and filter the input voltage. Figure 1 shows an output from a chart recorder.

For many chromatographers, the replacement of chart recorders with easily available and inexpensive integrators and data systems brought great relief from physically measuring peak heights and widths followed by manually calculating the peak areas. However, the advance of automating the data collection and calculation, essentially by a black box, brings problems in itself.

We should be asking questions such as:

- What does the A/D unit actually do?
- How does it affect my results?
- Am I in control?

But am I?

Using a data system, you are placing an interpreter between you and the chromatographic system. The interpreter is designed by a third party and places design constraints on the data-acquisition process. Let's look first at the heart of the matter, the A/D unit.

Essentials of A/D Conversion

Analogue-to-digital conversion is a process by which a continuously variable signal (e.g., analogue voltage) from an ultraviolet (UV) detector is converted to a binary number that can accurately represent the original data. These data are then passed to a computer for processing and storage. It is necessary to convert the analogue signal to a digitized form because conventional computer systems only handle numerical information in the form of a binary code comprising a series of zeros and ones.

A simple description of an 8-bit device is shown in Figure 2 and reference 3 and we can describe each section. We'll go through the process of data conversion in a number of simple steps.

Connecting the detector lead: The first stage of the A/D circuit is an adjustable gain amplifier, generally based on an operational amplifier with a non-inverting configuration to allow a high-input impedance. Wow! Sounds very impressive doesn't it? But what does this actually do? The amplifier takes the input voltage from the chromatographic detector and produces an output that is matched to the input of the A/D chip. For instance, many older high performance liquid chromatography detectors had an output range of 0–10 mV or 0–100 mV intended for chart-recorder input, therefore, if connected to a data system the amplifier boosts the detector signal to the standard input range (typically 0–1 V) of an integrated circuit A/D converter.

Look at the back of an A/D unit in your laboratory: there may be two or more input ranges. If your detector has a 0–1 V output, connecting the detector lead to the 0–1 V input on the box cuts out the amplifier and you can feed the detector signal directly to the A/D.

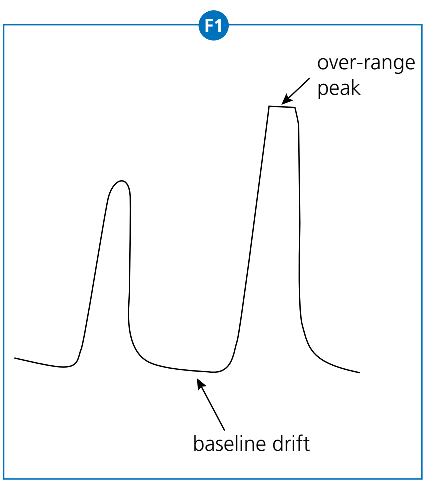

Figure 1: *Analogue output from a chart recorder.*

Converting the detector output to a number: When the appropriate control signal is applied to the A/D chip's "convert" pin, the analogue voltage at its input is converted into a digital value (0–255) that is stored in an internal buffer. The digital value can then appear as an 8-bit parallel digital signal on the output lines. There are eight output lines and each one carries one-eighth of the signal information.

By adjusting the amplifier's gain and offset voltage, an 8-bit A/D unit can be set to produce binary 0 (i.e., 00000000) output for 0 V input and binary 255 (i.e., 11111111) output for 10 mV input, binary 127 for 5 mV input, and so on.

Problems and constraints: (This may be one of the bits you may not want to read.) We don't wish to be "party poopers" but there are one or two problems and constraints on this process:

- Time and Sampling Rate: if you remember from the section on analogue data, the humble chart recorder produces a continuous, albeit smoothed, plot from the detector. An A/D unit cannot. The conversion process takes time, short from the perspective of a human but an eternity from that of an electronics engineer. With a balance between cost and availability, the highest sampling rate commonly available is about 100 Hz (points per second). For conventional liquid chromatography this sampling rate is much greater than needed, but for some capillary electrophoresis applications this may be just adequate.
- Resolution: the output signal accuracy is limited by the resolution of the converter. In the 8-bit example given above, if the input is a maximum of 10 mV, then this can only be represented by 256 values, that is, in steps of approximately 0.039 mV. Hence, it cannot discriminate between 0 and 0.01 mV. This also assumes that the device is perfectly accurate and has no noise (but you don't really believe this do you? — we'll be back to this topic later).

Therefore, what you will get out of the data system will be a series of steps, based on the voltage sampled and converted to numbers (Figure 3). Depending on the quality of your chromatography and the chromatography data system, you can get peak shapes that range from the early Egyptian step pyramids to the later classical ones at Giza that are every tourist's dream. This is what you get in EVERY data system, you just have to enlarge any peak to see it (alright go and do it — you really are a masochist aren't you?).

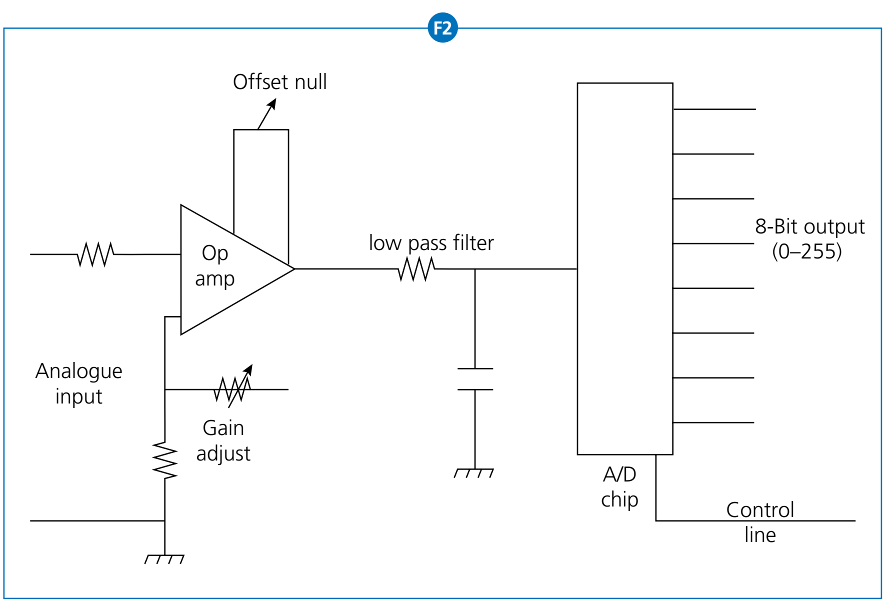

Figure 2: *8-Bit analogue-to-digital converter unit.*

A/D Converter Resolution

The two constraints, as outlined above, are the sampling rate and resolution of the A/D unit. The constraint that determines the quality of YOUR chromatography is the A/D unit resolution. This is controlled by the number of bits that the A/D can output. Table 1 gives a list for various bit lengths and the corresponding resolution that you can get.

So why is this important? This depends on the type of work that you do. If you are working within a narrow concentration or amount range, a low-resolution A/D may be suitable. There will be problems in discriminating between 97% and 100% of the nominal amount, but that's only minor. However, if you are determining peaks over a wide concentration range, say a main component and any related impurities, a low-resolution A/D will allow you to pass every batch of product because you won't be able to see the impurities!

So how much resolution do we need? On the face of it, as much of it as we can get! However, there are cost penalties, longer readout times and software issues, as well as noise and drift considerations to take into account. Chromatography data systems typically employ a minimum of 16-bit A/D converters. The situation is complicated by the fact that some 16-bit devices can be mathematically enhanced to 21 or 24 bit!

More Potential Problems: Multiplexers

Even 16-bit A/D devices are expensive, so the sharing of two or more chromatographs is common in many data systems using a multiplexer. A multiplexer is used for multichannel data acquisition from a single A/D converter. Its purpose is to sample each analogue input line in a fixed sequence,

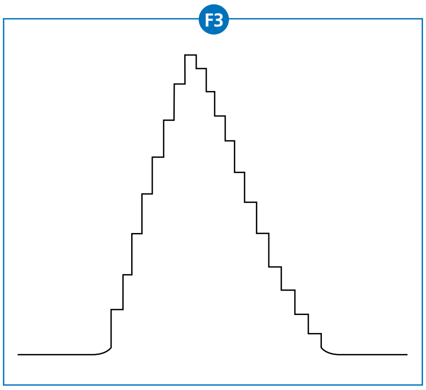

Figure 3: *Digitized peak after A/D conversion.*

allowing sufficient time on each channel for the A/D converter to acquire a portion of the analogue signal, convert it to digital and to pass the output to the computer. As an A/D converter takes several milliseconds to acquire a reading, several analogue inputs can be easily handled by a single multiplexer. However, the greater the number of channels connected, the less time is available for data acquisition.

Figure 4 shows how several chromatographs can be connected to an A/D unit via a multiplexer. The greatest advantage that a multiplexer data-collection system offers is one of economy, as several chromatographs can be connected to a system. However, the cost differential is eroded by the sophistication of the software required to acquire the data. The sampling rate and sequence of the multiplexer must be strictly controlled to maintain the integrity of the data from each individual channel. Whichever approach is used, the signal will have to travel a distance of several metres and background electronic noise will inevitably be generated, which can degrade the quality of the signal.

In addition, there is the problem of ensuring that data being acquired on one channel does not interfere with the data being obtained on another. This problem is known as crosstalk.

Approaches to User Qualification of A/D Converters

End users need to demonstrate fitness for purpose of their A/D boxes for some or all of the following parameters:

- Accuracy
- Sensitivity
- Precision
- Linearity
- Noise
- Drift
- Crosstalk.

We'll consider each of these topics.

Accuracy, Sensitivity & Precision

The role of the A/D converter is to accurately represent the analogue voltage from the detector in digital form. This accurate representation includes adequate precision.

Some chromatographers have argued in the past that it is unnecessary for the A/D converter to be accurate, but merely precisely wrong because concentrations are derived from peak-area or height ratios. This argument may equally be applied to other types of analytical measurement, for example, spectroscopic measurements for which a standard material is available.

The problem is that there appears to be a lack of internationally accepted standards for chromatographic A/D units. Or could it be that chromatographers are not interested in standards as it would mean more work? However, one has only to look at the European Pharmacopoeia's monograph on UV spectrophotometry (4) to see a specific requirement for absorbance and wavelength accuracy and precision, as well as other instrumental features. Therefore, the spectroscopist often has a better understanding of the key parameters for instrument performance than the chromatographer. However, the lack of recognized standards hampers the chromatographer from assuring his data quality.

Furthermore, the Food and Drug Administration (FDA) in the Good Manufacturing Practice regulations (5) specifically requires that, "The calibration of instruments, apparatus, gauges and recording devices at suitable intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event of accuracy and/or precision limits are not met. Instruments, apparatus, gauges and recording devices not meeting established criteria shall not be used."

ISO (6) goes further than this by requiring, "…the user shall identify, calibrate and adjust all inspection, measuring and test equipment and devices that can affect product quality at prescribed intervals, or prior to use, against certified equipment having a known valid relationship to nationally recognized standards."

Such is the impact of analytical data, particularly chromatographic data, on the establishment of product quality that the FDA requires test methods to be validated such that (7), "The accuracy, sensitivity, specificity and reproducibility of the test methods employed by the firm shall be established and documented."

Indeed, manufacturers specify the accuracy and precision of their interface boxes.

Table 1: Various Bit Lengths and Corresponding Resolutions.

A/D Bit Length	Resolution Elements (2^bit length)	Discrimination for 10 mV input in μV
8	256	39.0625
10	1024	9.765625
16	65536	0.152588
21	2097152	0.004768
24	16777216	0.000596

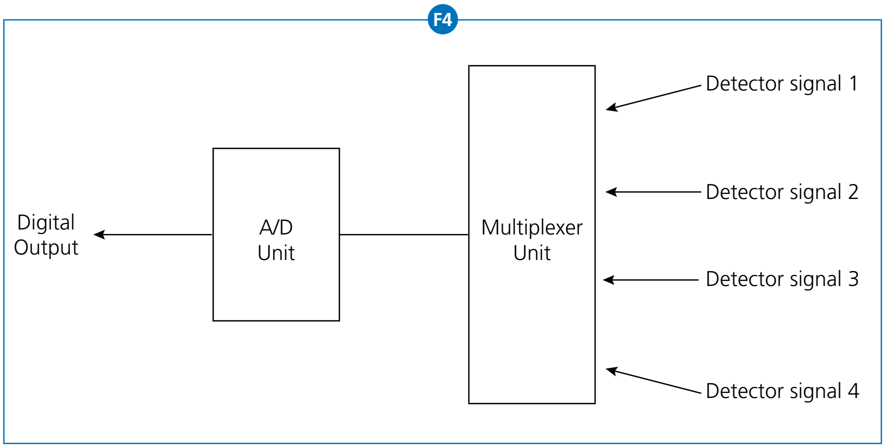

Figure 4: *Linking several chromatographs to a single A/D unit via a multiplexer.*

One manufacturer has published an approach to the validation of interfaces for chromatography data systems (8). The manufacturer concludes that, "To ensure the validity of the chromatographic results determined by computerized data-analysis systems, laboratories must have the means to evaluate the performance of A/D interfaces used by these systems."

The approach it employed involved the use of National Institute of Standards and Technology (NIST)-certified digital-to-analogue converter and standardized test patterns. Other signal generators are available but you must ensure that the signal quality is orders of magnitude better in terms of resolution and noise than the A/D device you are testing. One such device, CalPeak II, has an output signal quality that is equivalent to 54-bit resolution (2 parts in 10^{16}!). We shall see some of the results of a similar approach below.

The sensitivity issue is essentially corrected by the choice of A/D unit. It is most important to select the correct data system for your application. If you need to analyse trace impurities and major components you will need a more exacting specification than if you are only concerned with product release main peak assays.

A/D Accuracy and Precision in Practice

Figure 5 shows the results of challenging each of the 32 channels on a data system with 10 peaks of the same voltage. The source for this was a calibrated voltage source described in reference 8. Ten replicate Gaussian-shaped peaks, each having a maximum peak height of 815 mV, were input into each of the 32 A/D channels in turn.

The resulting peak heights calculated by the data system have been meaned and these figures have been plotted with their standard deviations to give an indication of the precision of the data. The measured µvolt peak heights are in the range of 812 000 to 816 000, compared with the input voltage of 815 000 µvolts. For the most part, the majority of the A/D units are accurate and very precise. But as you can also see from the figure, channel 1 is the exception as the mean voltage is near to 825 000 µvolts.

Is this difference (higher by approximately 1.2%) significant? Does it matter? This is obviously an area for further investigation. For instance:

- What is the uncertainty in the calibration of the voltage source?
- What is the specification for the accuracy of the A/D unit?
- Is this a unit that has degraded over time or has it always performed with this apparent bias (hence, the importance of performance qualification)? You would perform this test during operational qualification, wouldn't you?

In contrast, if you look at the means of the peak-area measurements for all of the A/D units the overall mean is very good and there does not appear to be any problem. The unit only appears to be a significant outlier when measuring peak height, because area (microvolt seconds) data appears much less sensitive to the actual maximum height. Note that the area precision is 100 times better than peak-height precision! Why do some chromatographers have such an affection for using peak heights one wonders?

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Table 2: Peak-Area and Peak-Height Measurements.

Parameter	Peak Area (µV*seconds)	Peak Height (µV)
Mean	6232887	814821
Min.	6217089	812269
Max.	6243955	824828
Standard Deviation	5582.6	1895.6
%RSD	0.09	0.23

F5

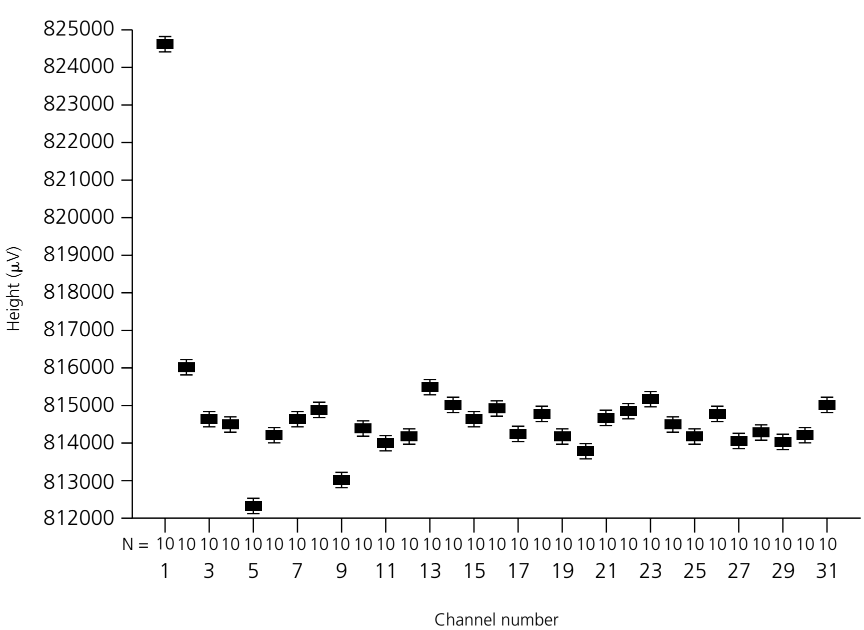

Figure 5: *A/D accuracy and precision in practice.*

Linearity

The one feature everyone agrees upon is that the A/D converter has to be linear! How linear and how to test it is not so well agreed. The best approach is to use a device such as described in the previous section covering the overall range used. This does mean the dynamic range of the A/D itself, which is usually better than the data system requires. For example, a common detector range accepted by a chromatography data system could be –100 to 1000 mV. However, the A/D itself might be designed to operate over –250 to 2500 mV.

Some systems will alert the user to the fact that the signal from the A/D unit has gone over range. This is an essential feature that should be mandatory on all data systems, but how many users actually request such a feature? Also an under-range indicator would be very useful to help interpret many chromatograms, especially those with negative peaks. Regardless of this, a user will need to establish that saturation does not occur nor that any negative voltages cause non-linearities.

There are no established or standard methods specifically for testing A/D linearity but a little known ASTM standard for testing fixed-wavelength photometric detectors in liquid chromatography has proved a useful approach (9).

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Table 3: Linearity of A/D Units (values are means for all 32 channels).

% Input	Measured Peak Area (µVolts*seconds)	Calculated Peak Area (µVolts*seconds)	% Deviation from 100% value
0.78125	48723.3	48696.4	0.06
1.5625	97495.3	97392.9	0.11
3.125	194893.5	194785.7	0.06
6.25	389746.2	389571.5	0.04
12.5	779341.7	779143.0	0.03
25	1558622.3	1558286.0	0.02
50	3117286.9	3116572.0	0.02
100	6233144.0	N/A	N/A
% Input	**Measured Peak Height (µVolts)**	**Calculated Peak Height (µVolts)**	**% Deviation from 100% value**
0.78125	6365.4	6362.2	0.05
1.5625	12741.3	12724.5	0.13
3.125	25469.6	25449.0	0.08
6.25	50936.7	50898.0	0.08
12.5	101857.2	101795.9	0.06
25	203717.4	203591.8	0.06
50	407451.6	407183.6	0.07
100	814367.3	N/A	N/A

Assessing A/D Linearity in Practice

A useful feature of calibrated voltage generators is the ability to produce peaks of increasing voltage and test the linearity of the A/D unit. Table 3 shows the linearity of a single channel.

There are eight peaks, each with a maximum input voltage and area half of the preceding one. The data system has calculated the peak height after conversion, shown in the second column of the table. The third column shows the calculated peak height value as a basis of a *pro rata* calculation of the 100% peak, this figure is just divided by two continuously. Comparing the corresponding figures in columns 2 and 3, the percentage deviation can be calculated, shown in the fourth column. As we can see, this A/D converter shows good linearity over the voltage range tested (0.815 to 0.0064 V).

However, there is a slight problem as the A/D unit itself has an input range of 0–1 V. Therefore, we are not testing the unit input voltage between 0.815 and 1 V. Does this matter? Yes, of course it does, as there is a need to show fitness for purpose over the entire operating range.

There is a design flaw with some of these voltage simulators, which is the lack of an over-range signal. We must have generators that are capable of testing the sensitivity of the A/D unit for a signal near, at and above the maximum input voltage. This will give us the confidence that the A/D unit has been properly designed and that when the signal exceeds the maximum input, the data system can inform the user.

Noise & Drift

These are most important for ongoing performance qualification as they are potentially more likely to degrade than the linearity as the electronics age. Again the ASTM standard provides an approach that can be adapted to address these concerns (9). Not all the noise comes from the detector and the A/D unit. Chromatographers need to be aware of the sources of noise and how to minimize them. A recent paper by Ouchi is a good starting point for information on these aspects (10). The book by Norman Dyson is also a very good source for further information on noise and data systems (11).

Crosstalk

This is probably the most neglected area of all when looking at A/D units. It is usually only an issue for multiplexed A/D converters. However, one of the most commonly found configurations is that each interface box will accept between two to four channels of input. From a data integrity standpoint, it is essential to ensure that the electronics and associated software are capable of keeping the channels totally separate.

One way to approach this problem is to acquire a chromatogram on one channel whilst saturating the other. Of course, the signal in the first channel will be completely unaffected by the second, won't it? You haven't tried it? Wouldn't it be a good idea to try to find out if your chromatography data system works?

Summary

The role of the A/D converter is critical in assuring data integrity. You must know and understand its advantages and limitations. These will impact on the quality of the derived analytical information. End users must assure themselves of both the adequacy of the A/D converter to perform the task required and to monitor its ongoing performance. Regulators are focusing more and more on the qualification of analytical equipment and the documented evidence for its suitability for use.

Large systems present special problems for overall system performance. Technical adequacy is not sufficient on its own. It must be capable of the response times required at capacity loading, otherwise it is not fit for purpose.

Acknowledgement

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References

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Dr Chris Burgess is Principal of Burgess Consultancy, Darlington, County Durham, UK. Dr Dianna G. Jones is President of SciOptics Corp., Orlando, Florida, USA.