Questions of Quality

Is Your Method Specific Or Just Selective?

In an earlier "Questions of Quality" column on method validation (1), we briefly discussed the term selectivity when applied to chromatographic methods. In this column we want to discuss selectivity in more depth and compare and contrast it with specificity. We will explain in more detail why selectivity is the correct term to use and why specificity is not, especially in the context of chromatographic methods of analysis. Yet despite this, some chromatographers, and even some industries, hide their heads in the sand and refuse to accept common sense and good analytical science, and continue to use the wrong terminology.

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Comparative and Absolute Methods of Analysis

Let's look at this problem of terminology first from the basis of measurement in analytical science rather than just from a narrow chromatographic perspective. A quantitative method needs a reference standard to calibrate the detector response. However, there are two types of methods of analysis: absolute and comparative (also called relative). Absolute methods of analysis are based on an absolute property of an analyte, for example, mass. In this instance, the response of the property can be used once the instrument is calibrated as it is assumed there is no interference from the reagents, matrix or impurities. The instrument is calibrated periodically and should be checked before the analysis, but the property is always consistent and predictable. Gravimetric analysis is a typical method using known masses that can be calibrated to national weights, which in turn can be calibrated to international standards. Laboratories can have their own calibrated masses of either F1 or E2 standards.
Analysing silver by precipitation can be a very accurate method of analysis but assumptions still have to be made about the nature of the reaction. Most importantly, are there any other ions present that can form insoluble silver salts and, thus, interfere? In contrast, a comparative (or relative) analytical technique involves a daily calibration and is used in cases in which there may be changes in the background, such as reagents or matrix, which may modify the analytical performance of the assay method. Chromatography is a comparative analytical technique.

Stationary and Dynamic Measurements

In addition to absolute and comparative analytical methods, we can also talk about stationary and dynamic measurements.

Stationary measurements: As the name suggests, we have a static environment for taking measurements of the sample. Examples of static measurement are ion-selective electrodes and spectrometric methods such as ultraviolet (UV) or near infrared. Let's consider UV spectrometric techniques. We can take a measurement at a single wavelength, multiple ones or over the whole spectrum. If there are enough wavelengths measured and the spectrum is very characteristic then we can identify the analyte. However, UV is usually not very discriminating and we may not be sure if the spectra is from one or many analytes. Somewhat better selectivity can be achieved with fluorescence determination. However, we can use mathematics and chemometrics to deconvolute and evaluate the spectrum to help identify and characterize the analyte(s) present. This is a good example of selectivity in the mathematical domain.
Dynamic measurements: In contrast, dynamic measurements are made continuously, usually with a flow separation (i.e., chromatography). This very flourishing area can be divided into detection selectivity and separation selectivity.

- **Detection:** Here the selectivity is influenced by the processes taking place under specified conditions in the detector. Interfering components can have effects on the detection reactions (either in liquid or gas phase).
- **Separation:** Here the interactions taking place between the analyte and the mobile and stationary phases can be influenced by changes in mobile-/stationary-phase composition, as well as from extraneous materials that change the character of the separation system.

Definitions

OK, now we have some background information for our discussion, let's look at

some definitions of specificity and selectivity in more detail.

Selectivity: The selectivity of an assay is a measure of the extent to which the method can determine a particular compound in the analysed matrices without interference from matrix components. A method that is perfectly selective for an analyte or group of analytes is said to be specific (2).

Specificity: The validation procedure should confirm the ability of the method to assess, unequivocally, the analyte in the presence of other components that may be expected to be present (e.g., impurities, degradation products and matrix components, etc). The validation studies needed depend on the use to which the method is to be put. Lack of specificity of an individual analytical procedure may be compensated for by other supporting analytical procedure(s) (3). This definition from the International Conference on Harmonisation (ICH) is restricted to pharmaceuticals including active substances and formulations. The important area of bioanalysis, so important in bioavailability studies, is completely neglected.

Consequences of the Definitions

Your first reaction when reading these two definitions may be to think, "so what?", or, "why the fuss?". At first reading, there appears to be no great difference between the two definitions. However we would like you to think again. An analytical method is specific by definition only when it measures the analyte without any kinds of interference. Regardless of the source of the definition this is true (2, 3).

However, how common are truly specific methods? Not very, is the simple answer. It is becoming clear to most chromatographers that such methods are rare to say the least. Sometimes the attribute "specific" has been graded as "highly specific". Unfortunately, the selectivity parameter tends to be abused like no other, more of which we will cover later in this column.

The International Union of Pure and Applied Chemistry (IUPAC) (4) and other scientific organizations have dealt with this issue and they state that specificity is the correct and preferred term to use as an expression, if a method is free from interferences and only determines the intended analyte. The International Union of Pure and Applied Chemistry also concludes that specificity is the ultimate of selectivity. This specificity cannot be graded.

In real life, it is common with interferences from related or extraneous components present in matrices of environmental, agricultural, chemical, pharmaceutical, clinical, forensic or other origin. Often it is not possible to fully avoid those contributions. However, the degree of interaction can often be estimated and this is the reason why analytical chemists speak about selective methods and use the term selectivity. By definition, the selectivity of a method refers to the extent to which it can determine particular analytes in a complex mixture without interference from other components in the mixture (2, 4).

Unfortunately, in many instances people do not realize and understand that there is a difference between selectivity and specificity. There are numerous examples from various fields, in which the major methodological problem involves interferences, and in which the use of selectivity and specificity is unclear. In particular, this is true for those areas in which trace analysis is required, such as in agricultural, forensic, environmental or bioanalytical studies. In the pharmaceutical field this terminology problem has notably been discussed at length during the recent efforts of harmonization. As analytical chemistry is involved in so many fields in our society, it would be appropriate to highlight the importance of choosing methods with a degree of selectivity that can give the correct answer in a given situation.

When looking at analytical chemical literature it is apparent that more and more articles discuss the selectivity of a presented method or technique in the terms of IUPAC. Sometimes both terms are used interchangeably, but the discrepancy becomes clear when "high" specificity is discussed. In particular, this is obvious for biochemical methods in which antigen–antibody reactions are used. Here cross-reactivity is often shown and a more appropriate term should instead be "group selectivity".

The aim of any analytical method is to determine an analyte or a couple of analytes without interferences from other components in the sample or in the sample matrix. The construction of a method is, therefore, made in such a way that the influence from the matrix or from interfering compounds is minimized as far as possible. In other words, the properties of the analyte are used to isolate the measured signal from other ones. The degree of sophistication of a method very much depends on the complexity of the sample matrix and on how many selectivity-generating steps have to be introduced. Today, it is evident that sooner or later the selectivity in a method does not suffice. This has paved the way for the use of hyphenated techniques in which the selectivity can stand tougher samples.

If a method is perfectly selective for an analyte or group of analytes it can be considered to be specific. A common and serious mistake is calling a method specific when it is only selective. This is especially true when dealing with chromatographic methods that are not absolute, but only relative methods of analysis. Specific analytical methods are very rare.

Chromatographic Methods

Chromatographic separation methods offer good possibilities to minimize interference from the sample matrix and other compounds. The degree of selectivity depends on the interactions of the analyte and other co-injected compounds with the stationary phase and the properties of the mobile phase. The establishment of chromatographic separations some 30 years ago opened up the realization that other analytical methods suffered from a lack of selectivity. Today, the need for high-selectivity analytical methods can often be satisfied by modern highly efficient chromatographic techniques.

Detection in Chromatography

Universal detectors, such as flame ionization in gas chromatography (GC) and UV in liquid chromatography (LC) and capillary electrophoresis, at least at low wavelengths, are used when the total composition of a sample is of interest (such as in the test for impurity patterns of chemicals and pharmaceuticals). Alternatively, selective detection is necessary, in particular, for trace analysis with detectors such as fluorescence and electrochemical in LC and electron capture and the thermionic-specific detector (nitrogen-phosphorus detector) in GC.

The most selective detection system is no doubt the mass spectrometer (single-ion monitoring or selected-ion monitoring) with possibilities to work in both the positive and the negative ion mode. Mass spectrometry (MS) detectors have replaced other types in many laboratories because the performance and reliability is so much better. The combination of efficient separation systems and detection principles adapted to the properties of the analytes will, in many instances, give satisfactory selectivity for the analytical methods. However, it is sometimes important to undertake peak-purity testing using diode array detectors with absorbance ratios or chemometric evaluation which can reveal interferences, and mass spectral detection is an even more powerful way.

Hyphenated Techniques

The access of analytical methods has grown tremendously in volume and, in particular, in degree of sophistication. By orthogonal combination of several methods, such as chromatography and spectrometry (hyphenation), it has become evident that samples are very often more complex than first realized. Isomers and homologues appear that we were not aware of previously. The first successful demonstration of this principle was the GC–MS method that appeared in the late 1960s and later on revolutionized trace analysis.

The increasing use of tandem MS reflects the substantial increase in selectivity that can be obtained with this technique. This selectivity is often so high that the work-up procedure can be simplified or omitted. More than one cycle to treat product ions in successive fragmentations (MS^n), as in ion-trap MS, gives an extremely powerful tool to obtain the correct information without interferences. Today, the combination of LC with tandem MS is probably the technique in which selectivity comes closest to the meaning of specificity. However, that is dependent on the matrix. Recent reports have shown clearly that even with this technique (LC–MS–MS) interferences can occur, especially if the LC part of the method is too short. This indicates that extraneous materials are influencing the ionization process. Anyhow, it has, at least, become apparent that, through the development of techniques inducing increased selectivity, very few methods are in fact specific and this is why we should be careful in using the term specificity when validating analytical methods. Selectivity is the preferred term because it is not absolute.

How to Abuse Selectivity

Selectivity is a method-validation parameter without units or dimensions. Selectivity can be graded (totally, highly, very, partially, etc), but this gradation is relative, usually in the eye of the developer of the chromatographic method.

In contrast, specificity cannot be graded as it is absolute, as shown in the definitions used, or abused by the majority of analytical scientists. This is the confusing point for many analytical scientists who try to harmonize between the two definitions. However, for certain methods, such as those using ion-selective electrodes, complexometric titrations and spectrometry there are possibilities to either calculate selectivity coefficients or to measure the contribution from interfering analytes. This lack of a common recommendation on how to grade selectivity is the start of the problem, especially when compared with other parameters, such as:

- precision (coefficient of variation or relative standard deviation)
- accuracy (percentage accuracy: measured versus actual concentration)
- limits of detection and quantification (amount or concentration versus signal-to-noise ratio).

Moreover, there are statistical experimental design schemes and approaches for these parameters.

You may remember earlier in this column, some methods were described as highly specific. This is an interesting concept, especially for a parameter that is unitless and dimensionless. Therefore, can we have gradations of selectivity? How can we accurately describe if a method is, "highly selective", or, "just selective"? Consider a method that may suffer from an intermittent interference: can we have high selectivity on Mondays and Friday and only just partial selectivity on Tuesdays, Wednesdays and Thursdays?

In contrast, selectivity is virtually dimensionless, although the percentage of interference is, in a way, a sort of grading of the selectivity (i.e., measuring the extent of interferences or combinations from non-analytes). It is rare (unknown?) to find any statistical experimental design to evaluate it. Bioanalytical guidelines for method validation (5) propose that the fluids from six individuals be used to assess selectivity of an assay. However, this figure was picked out of a mixture of, "what is the minimum we can do?", and, "that's a reasonable number". As Hooper (6) stated, there was no statistical evidence for selecting six as a number for evaluating selectivity.

However, the main problem from a chromatographic perspective is that measuring selectivity is not a single experiment run during method validation. During the application of any chromatographic method many parameters change, such as instruments, reagents, columns, standards and chromatographers.

Therefore, chromatographers must be vigilant and monitor some of the areas that could affect the selectivity of their method by the use of blank samples. This is a continuous process. Once started: you cannot stop. You should investigate potential sources of contamination over the lifetime of the application of the method. Therefore, the use of blanks, both reagent and matrix, is pivotal in this process (7).

Much has been written about other parameters of method validation, but little about selectivity. What is needed is further research and development into the concept of selectivity, and especially, experiments to determine and monitor selectivity during validation and application of the method. This affects all application areas (pharmaceutical, environmental, agricultural, forensic, etc). The scientific organizations dealing with analytical chemistry should investigate selectivity, in particular, how far does a chromatographer need to explore the related substances (degradation compounds or manufacturing impurities), metabolites, matrix components and different sources or batches of matrix, co-administered medication, etc? The existing confusion among harmonizing activities reflects this lack of conceptual acceptance. The scientific organizations should strive to make their message more clear and widespread.

Summary

As highlighted by Gary Christian, in his book on analytical chemistry, a clear distinction should be made between selective and specific (8). Few analytical methods including chromatographic ones are truly specific. The recommendation made by IUPAC that specificity is the ultimate of selectivity is, therefore, to be promoted and selectivity should be favoured in all cases in which good analytical chemistry is presented.

The bottom line is that when dealing with most chromatographic methods the use of the term specificity deludes both the chromatographer from the perspective of poor analytical science and the user of the data by making them believe they are getting more than is actually being delivered.

The authors welcome your comments on the Selectivity/Specificity debate.

References

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(Footnote on p.3).