Trends in Analytical Chemistry 81 (2016) 51–62

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Peak measurement and calibration in chromatographic analysis

Leonid D. Asnin *

Department of Chemistry and Biotechnology, Perm National Research Polytechnic University, Perm 614990, Russian Federation

ARTICLE INFO

Keywords:
Calibration
Chromatography
Quantitative analysis
Peak detection
Peak integration
Inverse calibration

ABSTRACT

The precision and accuracy of quantitative chromatographic analysis depends significantly on the quality of calibration. This review considers such aspects of the calibration procedure as design of a calibration experiment, choice of a calibration model, and regression analysis of calibration data. A brief description of the theory of chromatographic detection and modern approaches to data acquisition and processing is given in the context of the calibration problem.

Contents

1. Introduction

Calibration is a key concept of quantitative analysis, a bridge linking an instrument's response and the amount of an analyte in a sample. Surprisingly, this topic has not enjoyed much attention till the last decades of the 20th century. Guiochon and Guillemin wrote in 1988, "*...this topic [quantitative analysis] is almost completely neglected in treatises, books, handbooks or textbooks. It is rarely talked about at meetings, as if calibration were a dirty business and errors a plaque and not a topic worthy of scientific discussion*" [1]. The situation has been improved since then, with many research efforts devoted to problems of peak measurement and calibration. Unfortunately, this progress is poorly reflected in the review literature, except the issues of regression analysis [2,3] and terminology [4,5]. In textbooks, still this topic is either omitted from consideration or discussed in very basic terms [6,7]. In this context, the purpose of

* Tel.: +7 342 2391511; Fax: +7 342 2391511.
E-mail address: asninld@mail.ru

http://dx.doi.org/10.1016/j.trac.2016.01.006
0165-9936/

this review is to provide the analyst with state-of-the-art information on measuring and using calibration curves and relating problems. Fundamentals of different standardization techniques, e.g., the external or internal standard methods, or the addition method, are out of scope of this study. These are covered in almost any book on chromatographic analysis [1,6,7]. The discussion of calibration curve proper is preceded by the consideration of the problem of data acquisition and processing. This is made because none of the most reliable calibration methods makes sense if detector response is measured or interpreted incorrectly.

This paper is dedicated to the memory of Prof. Georges Guiochon, whose remarkable work on the theory of detectors, peak measurement, and new techniques of calibration inspired and directed research of many others.

2. Detector response

Most chromatographic detectors belong to one of the two classes: (i) nondestructive concentration detectors (such as the refractive index detector or the thermal conductivity detector) whose signal is proportional to the concentration of an analyte in the mobile phase and (ii) destructive mass sensitive detectors (such as the flame ionization detector or mass spectrometer (MS)) whose signal is proportional to the mass flow rate of an analyte to the detector cell. There are detectors that do not fit either category because their response factor depends on the mobile phase flow rate or their mode of functioning depends on the conditions in the detector cell as it happens for the electron capture detector [1].

For the concentration detector, the relationship between the detector response, S, and the concentration of an analyte in the detector cell, c, is given by

$$S - S_0 = f_c \cdot c \tag{1}$$

where f_c is the response factor and S_0 the background signal, that is, the signal generated without analyte molecules in the detector cell.

Integration of the concentration profile over the eluted volume V taking into account Eq. (1) gives

$$\int c\mathrm{d}V = \frac{F_v}{f_c}\int (S - S_0)\mathrm{d}t = \frac{F_v}{f_c}A \tag{2}$$

where F_v is the flow rate and A the area of the chromatographic peak. Note that Eq. (2) assumes the background signal is properly corrected so that $\int (S - S_0)\mathrm{d}t = A$.

Since the integral in the LHS of Eq. (2) is the amount q of injected analyte, this latter relates to the peak area as

$$A = \frac{f_c}{F_v}q \tag{3}$$

In the case of a mass sensitive detector, the response relates to the mass flow rate according to Eq. (4), f_q being the response factor:

$$S - S_0 = f_q \cdot \frac{dq}{dt} \tag{4}$$

This equation after integration with respect to time, t, yields

$$A = f_q \cdot q \tag{5}$$

Eqs. (3) and (5) show that the peak area is the response function corresponding to the injected amount. As long as peak height, H, is proportional to A, this characteristic can serve as the response function too.

2.1. Noise

Detector signal is subject to fluctuations commonly called "noise". These fluctuations can be of both deterministic and random nature. An example of the former is sinusoidal variation of the baseline caused by periodic movement of a piston in an HPLC pump. Both instrument and environment can be the sources of noise. Felinger [8] describes different types and sources of noise relevant to chromatography. In chromatography with MS detection, chemical noise resulting from mobile-phase impurities, stationary phase leaching etc can play an important role [9,10].

The noise amplitude is characterized by the signal-to-noise ratio, S/N. Meyer et al. [11] have proposed an empirical correlation relating the relative standard deviation (RSD) of the peak area to S/N: $\mathrm{RSD} = 0.58/(S/N) + 0.003$. This expression demonstrates that a very small fraction (0.3%) of the peak area error is explained by phenomena other than the signal noise such as the sample injection variability [12].

3. Data acquisition and processing

3.1. Sampling

Detector response is an analog signal. In order to be handled by a computer data acquisition system, it is digitized. The sampling rate or the number of points taken to represent the original signal is the most important parameter for accurate peak area determination. This value is chosen as a compromise between the necessity to represent accurately the detector signal, which is achieved with a high sampling rate, and the desire to decrease the effect of the baseline noise and to avoid large data files that requires a low acquisition frequency. Usually, the manufacturers of chromatographic equipment recommend a sampling rate of 20 to 30 data points per the narrowest peak in a chromatogram. A rigorous theoretical analysis suggests a requirement of 10 points per standard deviation of a Gaussian peak [13]. For fronting or tailing peak profiles or in the case of overlapping peaks still larger sampling frequency is required [8,14]. A chromatogram may contain peaks with very different widths. Then a sampling rate that is optimal for narrow peaks will not be that for broad peaks. Modern data acquisition software allows the user to change this parameter throughout a chromatogram according to peak width.

3.2. Noise reduction

This is a common wisdom yet supported by experimental evidence [15,16] that noise negatively affects the precision of peak area determination. Integrators of the pre-computer age had electronic filtering devices, which separated to a degree electronic noise from the useful signal. Nowadays, integrators are out of use except, perhaps, some portable chromatographs. More efficient yet flexible digital filtering methods [8] implemented into data acquisition software are employed to reduce noise. Nevertheless, manufacturers still provide an option of analog noise suppression through the choice of the detector time constant, τ, a time interval over which the detector signal is averaged to give one data point. The averaging results in the distortion of the peak shape and lost of the original data. The larger τ, the more profound is the smoothing effect, but on the other hand, the stronger the distortion of the peak shape, with a probable falsifying effect on the peak area. A general recommendation is to set the time constant to ≈ 1/20 the peak width at half-height ($w_{1/2}$) of the narrowest peak to be quantified. If a desired S/N ratio is not achieved, the chromatogram can be further refined by means of digital smoothing [17].