Considerations on the determination of the limit of detection and the limit of quantification in one-dimensional and comprehensive two-dimensional gas chromatography

Ján Krupčík [a,*], Pavel Májek [a], Roman Gorovenko [a], Jaroslav Blaško [b], Robert Kubinec [b], Pat Sandra [c]

[a] Institute of Analytical Chemistry, Faculty of Chemistry and Food Technology, STU, Bratislava, Slovak Republic
[b] Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic
[c] Research Institute for Chromatography, Kortrijk, Belgium

A R T I C L E   I N F O

A B S T R A C T

Methods based on the blank signal as proposed by IUPAC procedure and on the signal to noise ratio (S/N) as listed in the ISO-11843-1 norm for determination of the limit of detection (LOD) and quantitation (LOQ) in one-dimensional capillary gas chromatography (1D-GC) and comprehensive two-dimensional capillary gas chromatography (CG×GC) are described in detail and compared for both techniques. Flame ionization detection was applied and variables were the data acquisition frequency and, for CG×GC, also the modulation time. It has been stated that LOD and LOQ estimated according to IUPAC might be successfully used for 1D-GC–FID method. Moreover, LOD and LOQ decrease with decrease of data acquisition frequency (DAF). For GC×GC–FID, estimation of LOD by IUPAC gave poor reproducibility of results while for LOQ reproducibility was acceptable (within ±10% rel.). The LOD and LOQ determined by the S/N concept both for 1D-GC–FID and GC×GC–FID methods are ca. three times higher than those values estimated by the standard deviation of the blank. Since the distribution pattern of modulated peaks for any analyte separated by GC×GC is random and cannot be predicted, LOQ and LOD may vary within 30% for 3 s modulation time. Concerning sensitivity, 1D-GC–FID at 2 Hz and of GC×GC–FID at 50 Hz shows a ca. 5 times enhancement of sensitivity in the modulated signal output.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Method validation is of utmost importance in the analytical laboratory to ensure that reliable analytical procedures are used under defined conditions. It is internationally recognized as an essential part of a comprehensive quality assurance system in analytical chemistry. In order to demonstrate that a method is suitable for its intended purpose, it must meet certain performance characteristics. The most important of them include applicability, specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness. LOD and LOQ are two fundamental elements of method validation that define the limitations of an analytical method [1–3].

Capillary gas chromatography (GC) is the technique of choice for analysis of volatile and semi-volatile solutes and is commonly performed on a single separation column (one-dimensional GC or 1D-GC). Determination of LOD and LOQ values is a typical requirement in 1D-GC for accreditation purposes. Comprehensive two-dimensional GC or GC×GC is a technique designated for the separation of very complex samples of volatile and semi-volatile compounds. In GC×GC, two columns of different selectivity are coupled in series. The sample is firstly separated on the first column (^{1}D column) and very small fractions of its effluent are then continuously focused via a modulator and then subsequently injected in and separated by the second column (^{2}D column). The effluent from the second column is monitored by a detector. Through modulation, an analyte eluting from the ^{1}D column fragments into several peaks which are much narrower than those eluting from the first column. Consequently, sensitivity in GC×GC is higher than in 1D-GC. GC×GC is more complex compared to 1D-GC and several sources of uncertainties are not yet quantified and no standard procedure for the determination of LOD and LOQ has been elaborated until now.

There are three most often used conceptual methods regarding LOD and LOQ, each providing a somewhat different definition. Principles of these methods are based on: (i) the blank signal, (ii) the signal-to-noise ratio (S/N) and (iii) the statistical data of

a calibration curve [1–21]. Depending on the definition chosen, the values of LOD and LOQ can vary greatly making it difficult for comparative purposes [6,7].

The aim of this study is to discuss the procedures based on the blank signal and the S/N for the determination of LOD and LOQ in 1D-GC and in CG×GC both with flame ionization detection (FID). The dependence of LOD and LOQ on the data acquisition frequency (DAF) of the FID for low boiling hydrocarbons is presented in detail for 1D-GC–FID and GC×GC–FID. The modulation time in GC×GC was varied between 1 and 5 s.

2. Theory

From the different conceptual methods regarding LOD and LOQ, the two methods based on the IUPAC [2] and on the ISO11843-1 [3] guidelines are discussed.

2.1. Method based on the standard deviation of the blank

The IUPAC document [2] defines LOD and LOQ as the concentration, c_L, or the quantity, q_L, derived from the smallest measure, y_L, that can be detected or quantified with reasonable certainty for a given analytical procedure.

The value of y_L is given by the equation:

$$y_L = \bar{y}_b + k \times s_b \tag{1}$$

where $\bar{y}_b$ is the mean value of the blank measures, s_b is the standard deviation of the blank measures, and k is a numerical factor chosen according to the confidence level desired [5–7].

Reliability in this context refers to accuracy and precision of the method. Both of these have to be high enough based on the objective of the measurement. Reliability also refers to the absence of any false signals. It is of no use that a method can detect minute amounts of a compound if there are interferences from other compounds that cannot be distinguished from the compound of interest. Thus the initial statement contains another, quite different goal of equal importance namely specificity. A detection limit is an exact, quantitative measure, whereas specificity is a more qualitative expression describing nonrandom events [5–7]. A statistical analysis for the detection limit is possible because only random fluctuations are considered in the calculation. As a consequence, a detection limit becomes meaningless if there are nonrandom sources of errors. One of the most frustrating sources of nonrandom distortions are interferences by other compounds. The detection limit only measures the ability of an instrument to separate a signal from noise. It has absolutely nothing to do with the assignment of the signal to a certain chemical. Again, the fluctuation in the instrument background is the only quantity needed to establish a detection limit in a signal domain [5–7]. If one wants to express this limit in concentration or mass terms, one has to convert signal units to analytical data.

Conversion of measurement units, y, to concentration, c, is performed for concentration dependent detectors by a calibration procedure and calculated from the formula:

$$c_L = \frac{y_L + \bar{y}_b}{b_c} \tag{2}$$

in which b_c is the sensitivity, a slope of the linear calibration curve $y = a_1 + b_c \times c$ where a_1 is an intercept.

Conversion of measurement units, y, to mass, m, for mass-flow-selective detectors is similarly performed by a calibration procedure and calculated from the formula:

$$m_L = \frac{y_L + \bar{y}_b}{b_m} \tag{3}$$

where b_m is the sensitivity, a slope of the linear calibration curve: $y = a_2 + b_m \times m$ where a_2 is again an intercept [5–7].

The IUPAC document [2] recommends to apply $k = 3$ and $k = 10$ for the calculation of LOD and LOQ, respectively.

Considering these recommendations the following equation is used to calculate the LOD for concentration selective detectors:

$$LOD = \frac{(y_{LOD} - \bar{y}_b)}{b_c} = \frac{3 \times s_b}{b_c} \tag{4}$$

Limit of quantification is then calculated by formula:

$$LOQ = \frac{(y_{LOQ} - \bar{y}_b)}{b_c} = \frac{10 \times s_b}{b_c} \tag{5}$$

Similar equations may be used for the calculation of LOD and LOQ for mass selective detectors [5–7]:

$$LOD = \frac{(y_{LOD} - \bar{y}_b)}{b_m} = \frac{3 \times s_b}{b_m} \tag{6}$$

$$LOQ = \frac{(y_{LOQ} - \bar{y}_b)}{b_m} = \frac{10 \times s_b}{b_m} \tag{7}$$

2.2. Method based on signal-to-noise ratio

ISO 11843-1 [3] and several official institutions [8–10] recommend to express LOD and LOQ for chromatographic analysis by a signal-to-noise ratio (S/N) criterion which is calculated by the formula [3,4]:

$$S/N = \frac{2 \times H}{h} \tag{8}$$

where H is the height of the neat peak corresponding to considered analyte, h is the range of the blank noise or the background noise situated equally around the place where the analyte occurs. Fig. 1 illustrates the principle of calculation of S/N on 1D-GC (A) and GC×GC (B) chromatograms. A signal-to-noise ratio (S/N) of three is generally accepted for estimating LOD and signal-to-noise ratio of ten is used for estimating LOQ [3,8–10].

3. Experimental

3.1. Samples

Toluene, n-nonane, n-decane, n-undecane and n-dodecane were obtained from Sigma–Aldrich, Buchs, Switzerland. Three model mixtures containing 25 μg/mL, 50 μg/mL and 75 μg/mL of each compound in chloroform (Sigma–Aldrich) were prepared.

3.2. Instrumentation

A TRACE GC×GC system GC (Thermo Fisher Scientific, Waltham, MA, USA) was used to perform comprehensive 1D-GC and GC×GC. The system was provided with a dual-stage CO_2 jet modulator. A fast flame ionization detector (FID) was used as detector, capable of producing a digital signal at a sampling rate up to 200 Hz. The injection system was a low dead volume split/splitless (SSL) injector containing a 105 mm × 5 mm i.d. glass liner. The first dimension column was a DB-5 (Agilent Technologies, Waldbronn, Germany) 30 m long, with an internal diameter of 0.25 mm and a phase thickness of 0.25 μm. The ^{1}D column was connected through a deactivated press-fit (Restek, Bellefonte, PA, USA) to a DB-WAX ^{2}D column (Agilent Technologies), 0.8 m long with an internal diameter of 0.1 mm and a phase thickness of 0.1 μm. 1D-GC was performed by operating the system without modulation. For GC×GC, modulations of 1, 2, 3, 4 and 5 s were applied.

3.3. Operating conditions

The oven temperature was programmed from 40 °C (2 min isotherm) at 2 °C/min to 160 °C. Helium of 99.996 purity (Linde Gas