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Estimate of gas chromatographic blanks Application to detection limits evaluation as recommended by IUPAC[1]

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Abstract

A new method to obtain the signal associated with a chromatographic blank is presented for inclusion within calibration procedures. Signal assigned to the blank is obtained by direct integration of the background noise by using extrapolated values of the base-peak width at concentrations different to the 'zero concentration'. Thus, detection limits which are better adjusted to a statistical evaluation are implemented. These limits are more in line with IUPAC recommendations.

Keywords: Detection limits; Calibration methods; Lauric acid; Stearic acid; Arachidic acid

1. Introduction

Gas chromatographic calibrations are characterized by the absence of analytical signals related to the blank. This implies that predictions in the lowest region of the calibration line are often made on extrapolated values not related to experimental checks on the blank.

In order to by-pass this problem, checks were made with a lot of samples just around the lowest concentration of analyte. However, this breaks the symmetry of the calibration method as the equidistance of analyte concentrations tested was broken since there were a lot of checks against the lowest limits of concentration.

Statistical tests were also distorted since there was a significant statistical weight coming from that part of the spectrum where the measurements were carried out (i.e. linearity [1]).

Moreover, the absence of signal from the blank prevents an estimate of the detection limit (DL) as recommended by IUPAC for spectrochemical methods of analysis ($DL = 3 \cdot S_b/b$) [2]. Therefore other methods have been used to try to overcome difficulties such as the S/N [3,4], minimum estimated amount [5], use of the independent term of the calibration equation [6], calculations on error propagation [7] or approximate estimations based on extrapolations of the calibration line [8].

However, all these methods are subject to practical difficulties. For instance, estimates based on the widely utilized signal to noise ratio (S/R) [9] lack

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suitable statistical tests to ensure the quality of the measured values. Thus, there is a lack of statistical information for a statistical comparison of the detection limits obtained by this method.

The minimum estimated amount is a difficult parameter to determine, as it also requires the signal to noise ratio, and no studies have been carried out to establish its statistical significance.

The use of the error propagation method leads to over-estimates of the detection limit while the use of the independent term from the calibration line deprives the IUPAC definition of statistical meaning [10]. The estimates based on considerations extrapolated from the experimental domain also have problems derived from this fact.

Here we propose a method to evaluate the signal from the blank using the background noise from the baseline of the chromatogram. Some information on the blank signal is superimposed onto the normal noise from the background, because, when chromatograms of samples with low concentrations are recorded, the width of the decreasing signals which are registered as a chromatogram tends towards a permanent value regardless of decreases in concentration.

2. Theory

2.1. Calculation of the width W_b of a chromatographic peak

The base width, W_b, of a chromatographic peak can be estimated in a straightforward manner from a chromatogram by estimating the initial and final times on the chromatogram baseline by an adequate choice of integration parameters. Obviously, the method can lead to an important random error since evaluations are carried out in a region where the uncertainty caused by the background noise interacts strongly with the measured values [11,12].

We can by-pass the problem by using the half-width for the peak height $W_{0.5h}$ as a parameter for width evaluation and then estimating the base width W_b. We can assume that the chromatographic peak shape is a Gaussian-type one [13].

The asymmetry of real chromatographic peaks has led to the use of the so-called 'exponentially modified Gaussian curves' (EMG) [14,15], as the use of the exclusively Gaussian model led to important errors in the characterization of chromatographic peaks [16].

However, provided that the asymmetry of the peak is not too high, errors for peak-area or variance are not important [17] and we will use this hypothesis as our method of calculation.

For a Gaussian model adjusted to describe a chromatographic peak (Eq. 1):

$$h = \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}\frac{(t-t_R)^2}{\sigma^2}} \quad (1)$$

where h is the peak height for a determined time t, t_R is the retention time and σ is the peak variance. Particularising Eq. (1) for peak parameters (see Fig. 1) the following value for the variance can be obtained (Eq. 2):

$$\sigma = \frac{W_{0.5h}}{2} \sqrt{\frac{1}{2 \ln \frac{1}{\sqrt{2\pi}\, h_{0.5}}}} \quad (2)$$

where $W_{0.5h}$ is the half-width of the peak and $h_{0.5}$ the half-height of the peak whose normalized value is 0.1995.

Estimate of W_b for 99.73% of the peak-area is then (Eq. 3) [18]:

$$W_b = 6\sigma = 2.548\, W_{0.5h} \quad (3)$$

2.2. Calculation of the width at the base W_{b0} at 'zero concentration'

Extrapolation of the graphs of $W_{0.5b}$ at different concentrations of analyte can give us an adequate

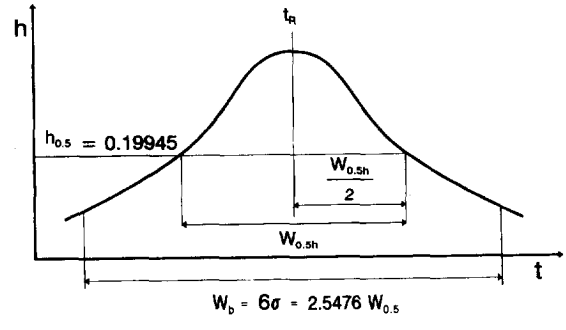

Fig. 1. Characteristic parameters of a Gaussian peak. W_b, base peak width; $W_{0.5h}$, half-width peak; $h_{0.5}$, half-height peak; t_R, retention time; σ, peak variance.

statistically significant idea of the width of the base for 'zero concentration' (estimate of the blank).

The adequate values of W_{b0} are then those given by Eq. 3.

2.3. Measurement of the signal coming from the chromatographic blank

The blank signal for each analyte can be determined by integration over the baseline of the chromatograms taking a width $t_R \pm 0.5W_{b0}$ where t_R is the retention time of the analyte and W_{b0} has been evaluated as explained above.

Each experimental measurement requires a new injection and a new chromatogram in order to obtain adequate information on the blank.

2.4. Test for the signal 'measured' from the chromatographic blank

To check that the measured values are compatible with the rest of the signals obtained during a calibration it is necessary to obtain a 'non-significant' conclusion when the test for 'lack of fit' is carried out [1].

Carrying out the test requires that the calibration is made obtaining several replicates, but if this is not possible, so-called 'robust regression techniques' [19,20] can be used.

3. Experimental

3.1. Apparatus

All chromatographic measurements were performed with a Hewlett-Packard 5890A GC fitted with a HP 7673A automatic injector and an automatic sampler, a HP 5971A MS spectrometer and a HP DA-5100 data system. Injection port liners of GC 78.5×2 mm were made of quartz and silylated before use. The column was a HP-5 MS fused silica capillary (30 m×0.25 mm I.D., 0.33-μm film thickness) coated with cross-linked 5% phenyl-methyl silicone gum phase.

3.2. Reagents

All reagents (Sigma, St. Louis, MO, USA) were of analytical-reagent grade unless stated otherwise.

3.3. GC–MS conditions

The sample was introduced into the injection port using an automatic injector. The operation mode was splitless with a 2-min venting time and a 200°C injector temperature. Helium was used as the carrier gas at a column flow-rate of 1 ml/min, a purge vent flow-rate of 50 ml/min and split purge flow-rate of 2 ml/min. The inlet pressure was 105 KPa. The temperature program of the oven started at 75°C (for 1 min) and increased at the rate of 30°C per minute to a final temperature of 270°C. This temperature was held for 7 min.

The electron multiplier (EM) voltage was between 1750 and 1950 V, the transfer line was kept at 280°C. The GC–MS was periodically autotuned with perfluorotributylamine (PFTBA) with an ionization energy of 70 eV.

4. Results and discussion

Fig. 2 shows a graph of $W_{0.5h}$ vs. concentration for the methyl esters of the lauric, stearic and

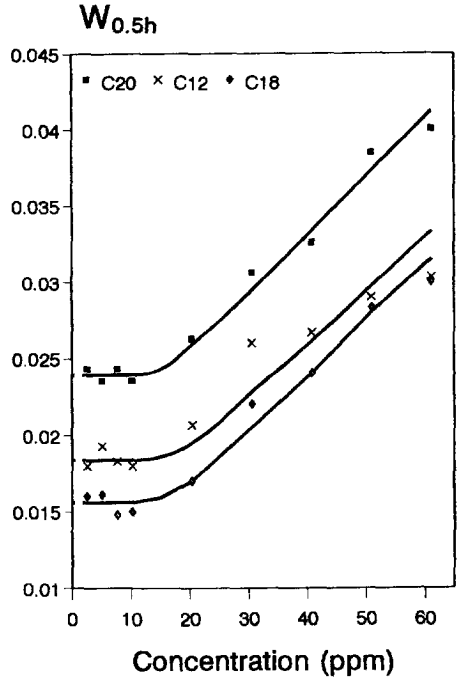

Fig. 2. Base-width peak vs. analyte concentration. C_{20}, arachidic acid; C_{12}, lauric acid; C_{18}, stearic acid.

Table 1
$W_{0.5h}$ and W_{b0} extrapolated to 'zero concentration' of analyte

Analyte	$W_{0.5h}$	W_{b0}	$t_R \pm 0.5W_{b0}$
Methyl laurate	0.0194	0.0494	5.401±0.025
Methyl stearate	0.0155	0.0396	7.671±0.020
Methyl arachidate	0.0240	0.0610	9.503±0.031

arachidic acids while Table 1 contains the $W_{0.5h}$ values at 'zero concentration' and the integration limits used to get the 'signal from the blank'.

At first sight, to get the blank it could be desirable to take all the measurements for all the analytes on a single chromatogram in order to avoid a large number of injections. What we should do to achieve this is to get the different measurements for each analyte integrating with base-width corresponding to different retention times along the same chromatographic blank. Further, to have reliable results, background noise must be relatively uniform along the chromatogram and there should be no offsets of the background signal.

To check the efficiency of the methodology devised here, we carried out an experimental design based on a design with 'latin squares' [21,22]. The variables of the trial are: analyte, A; base width at 'zero concentration', W_{b0}; and retention time, t_R. Table 2 indicates the structure of the trial and the values measured (3 replicates were taken for each analyzed value).

The analysis of variance of the data in Table 3 shows clearly that there are significant differences between the measured areas for a same analyte at different retention times t_R (significance level $P < 0.05$). Tukey's range test show differences in the comparisons between all the pairs of such areas [21,23].

But, this is just our case because there are

Table 2
Area of chromatographic 'blanks' calculated from a latin squares design

		W_{b0} (L)		W_{b0} (E)		W_{b0} (A)
		154 290		78 590		239 300
Blank 1	t_R (L)	177 950	t_R (E)	64 650	t_R (A)	253 540
		226 680		78 100		238 100
		106 260		102 270		281 760
Blank 2	t_R (E)	101 110	t_R (A)	131 260	t_R (L)	309 720
		125 780		109 120		383 920
		187 650		121 550		125 250
Blank A	t_R (A)	150 650	t_R (L)	149 270	t_R (E)	122 280
		164 920		207 130		214 660

W_{b0}, base-width peak at 'zero concentration'; t_R, retention time. L, E and A are the methyl esters of the lauric, stearic and arachidic acids, respectively.

Table 3
Analysis of variance of Table 2 data

Parameter	*SS*	*d.f.*	*MS*	*F*-ratio	*P*
Blank	0.00245	2	0.00123	0.949	0.404
Width	0.07370	2	0.03685	28.520	0.000
Time	0.05550	2	0.02775	21.477	0.000
Residuals	0.02584	20	0.00130		
Total	0.15750	26			

SS, sum of squares. *d.f.*, Degrees of freedom. *MS*, mean squares. *P*, significance level.

significant differences in the retention time t_R. Therefore, it is not sufficient to use a single chromatogram for all the blanks and it is necessary to have an injection for each replicate. But the results can not be extrapolated to every case and checks need to be made beforehand.

4.1. Calibration

Equally spaced standard dilutions of lauric, stearic and arachidic acids were previously esterified with boron trifluoride [24]. The analytical signal was taken as the ratio between the areas of the analyte and the internal standard of dodecanoic acid at 10 mg l^{-1}. Two replicates and three injections were carried out. To the data thus obtained were added the signals from the 'chromatographic blank' of six different chromatograms obtained as described above from 'zero concentration solutions' of the analyte (see Table 4).

The statistical parameters for each calibration are given in Table 5. Values of the standard deviation at 'zero concentration' have been calculated from the calibration data using the equation derived from linear regression [8].

4.2. Detection limit

The recommended IUPAC detection limit for spectroscopic methods is to take three times the standard deviation of the signal assigned to the 'zero concentration' as the signal threshold to indicate the presence of an analyte. This standard deviation assigned to 'zero concentration' is obtained from a

Table 4
Analytical signals obtained for the different calibration curves

Lauric acid		Stearic acid		Arachidic acid	
ppm	signal	ppm	signal	ppm	signal
0.000	0.0013	0.000	0.0004	0.000	0.0011
0.000	0.0011	0.000	0.0006	0.000	0.0014
0.000	0.0011	0.000	0.0008	0.000	0.0002
0.000	0.0008	0.000	0.0003	0.000	0.0013
0.000	0.0009	0.000	0.0004	0.000	0.0002
0.000	0.0010	0.000	0.0005	0.000	0.0013
0.252	0.2230	0.314	0.3210	0.260	0.1520
0.252	0.2280	0.314	0.3280	0.260	0.1540
0.252	0.2310	0.314	0.3210	0.260	0.1530
0.252	0.2100	0.314	0.2960	0.260	0.1350
0.252	0.2120	0.314	0.3150	0.260	0.1560
0.252	0.2080	0.314	0.3230	0.260	0.1390
0.504	0.4290	0.628	0.5790	0.520	0.2970
0.504	0.4310	0.628	0.5540	0.520	0.2930
0.504	0.4320	0.628	0.6090	0.520	0.3130
0.504	0.4470	0.628	0.6060	0.520	0.3420
0.504	0.4520	0.628	0.5940	0.520	0.2930
0.504	0.4440	0.628	0.6510	0.520	0.3350
0.756	0.6660	0.942	0.9010	0.780	0.4850
0.756	0.6700	0.942	0.8940	0.780	0.4730
0.756	0.6710	0.942	0.9220	0.780	0.5130
0.756	0.6510	0.942	0.8860	0.780	0.4800
0.756	0.6580	0.942	0.8810	0.780	0.4620
0.756	0.6620	0.942	0.8330	0.780	0.4630
1.008	0.8730	1.256	1.2170	1.040	0.6660
1.008	0.8820	1.256	1.1810	1.040	0.6340
1.008	0.8850	1.256	1.2310	1.040	0.6690
1.008	0.8930	1.256	1.1950	1.040	0.6490
1.008	0.8970	1.256	1.1720	1.040	0.6510
1.008	0.8910	1.256	1.1840	1.040	0.6400

Table 5
Statistical parameters of calibrations shown in Table 4

Statistical parameter	Lauric acid	Stearic acid	Arachidic acid
s ($\times 10^2$)	0.789	2.321	1.559
a ($\times 10^3$)	−1.447	7.666	−8.050
s_a ($\times 10^3$)	2.495	7.340	4.932
b	0.879	0.943	0.628
s_b ($\times 10^3$)	4.042	9.544	7.744
P_{LOF}	63.8%	27.7%	14.3%
s_{Co} ($\times 10^2$)	0.463	1.289	1.274

s, standard deviation of residuals; a, independent term; s_a, standard deviation of independent term; b, slope; s_b, standard deviation of slope; P_{LOF}, 'lack-of-fit' F-test; s_{Co}, standard deviation to 'zero' concentration.

Table 6
Detection limits calculated from different models

Model	Lauric acid	Stearic acid	Arachidic acid
Approximated[a]	0.029	0.080	0.073
S/N[b]	0.136	0.129	0.119
This paper	0.014	0.039	0.038

[a] Calculated from Eq. 3. $[(n-2)/(n-1)]^{1/2} s_{Co}$ [8].
[b] Calculated from a signal three times the background noise.

previous estimate of the slope of the calibration curve, and then the ratio (standard deviation of the blank)/(estimated slope) is calculated.

Here, at 'zero concentration', because of the method, the standard deviation measured is that of the background noise and not that of the analytical signal itself. The latter must be calculated from the equations for regression. This implies homoscedasticity in the variances and one gets values which describe the variability of the blank more adequately.

Table 6 gives different values of the detection limit deduced from this work compared with those obtained by the approximate method and those obtained from the signal/background ratio. We can see that the detection limits calculated from the S/N are higher than those calculated by other methods. This is obvious as the limit is calculated from peak-heights and since the $W_{0.5h}$ value remains constant at low concentrations of analyte (see Fig. 2) and, in general, peak-heights against the baseline need to be rather high to be visually detected.

Thus, the area determination by integration method discussed in this paper provides for discrimination of analyte values below lower limits. It is because of this that the statistical detection limits are then smaller.

Moreover in the approximate method by extrapolation one is purposely getting away from the experimental values while in our method one is using 'experimental' values to get the limit value.

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