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Some observations on the standard addition procedure in gas chromatographic analysis

C. Nerín*, J. Cacho, A.R. Tornés, I. Echarri

Dept. Química Analítica, Centro Politécnico Superior, Universidad de Zaragoza, 50015 Zaragoza, Spain

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Abstract

A critical study was made of the standard addition procedure as applied in gas chromatography. The general procedure when an electron-capture detector is used in gas chromatography is discussed with the example of the determination of organochlorine compounds in a certified reference material of animal diet.

1. Introduction

The gas chromatographic (GC) determination of organochlorine compounds in real samples often shows strong matrix effects. Usually these matrix effects are attributed to the interaction between the detector and other unidentified organic compounds contained in the sample. When these problems appear, the use of the standard addition procedure is suggested as a good alternative to avoid the matrix influence. However, the problems cannot always be eliminated in practice.

Analysis with different gas chromatographic detectors has been considered by some workers in an additional attempt to compare the responses obtained with various compounds, so that the interferences can be identified and eliminated.

Further, numerous theoretical and practical problems can be identified when the standard addition procedure is applied. The linear range and the slope of the straight line obtained can vary depending on the concentration level of the analytes. In addition, the treatment of the data obtained and the final plot selected, *i.e.*, response obtained *versus* concentration added or measured concentration *versus* concentration added, can modify the final results.

This paper presents a critical study of the standard addition procedure as applied to a certified sample (CRM 115) of organochlorine pesticides in animal diet. Several limitations of the standard addition procedure applied to real samples when using gas chromatography with electron-capture detection (ECD) are discussed.

2. Experimental

2.1. Apparatus and reagents

A Hewlett-Packard 57980 Series II gas chromatograph equipped with an electron-capture detector, a Selecta vibrator, a Heidolph rotary evaporator and Selecta ultrasonic bath were used.

α-Hexachlorocyclohexane, β-hexachloro-

* Corresponding author.

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cyclohexane, δ-hexachlorocyclohexane, hexachlorobenzene, γ-chlordane, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, α-endosulfan, *p,p'*-DDE, *p-p'*-TDE, *o,p'*-DDT and *p,p'*-DDT were obtained from Riedel-de Häen. Dichloromethane, light petroleum, hexane and cyclohexane (residue analysis quality) were purchased from Merck. Florisil and silica gel for residue analysis were supplied by Fluka and anhydrous sodium sulphate by Panreac.

2.2. Procedure

A 2.5-g amount of a certified sample (CRM 115) of animal diet was weighed exactly. This sample was placed in a 125-ml Soxhlet apparatus with dichloromethane–light petroleum (1:4). After 6 h of extraction at 35°C, the organic extract was transfered to a glass column containing a combined solid bed of 2.5 g of Florisil (7% deactivated) and 2.5 g of silica gel (7% deactivated). A 2-cm layer of anhydrous sodium sulphate was placed on the top of this column to keep the extract dried. After passing through the clean-up column, the organic extract was evaporated to 2 g and then analysed by GC–ECD.

2.3. Chromatographic conditions

The GC analysis was carried out with a fused-silica column (60 m × 0.25 mm I.D.) containing DB 1701 bonded phase with a 0.25-μm film thickness. The column oven temperature programme was as follows: 60°C for 2 min, increased at 20°C/min to 185°C, held for 10 min at 185°C, then increased at 5°C/min to 250°C and held for 20 min at 250°C. The injector temperature was 250°C. Hydrogen was used as the carrier gas at a flow-rate of 1.45 ml/min and nitrogen as the make-up gas at a flow-rate of 60 ml/min.

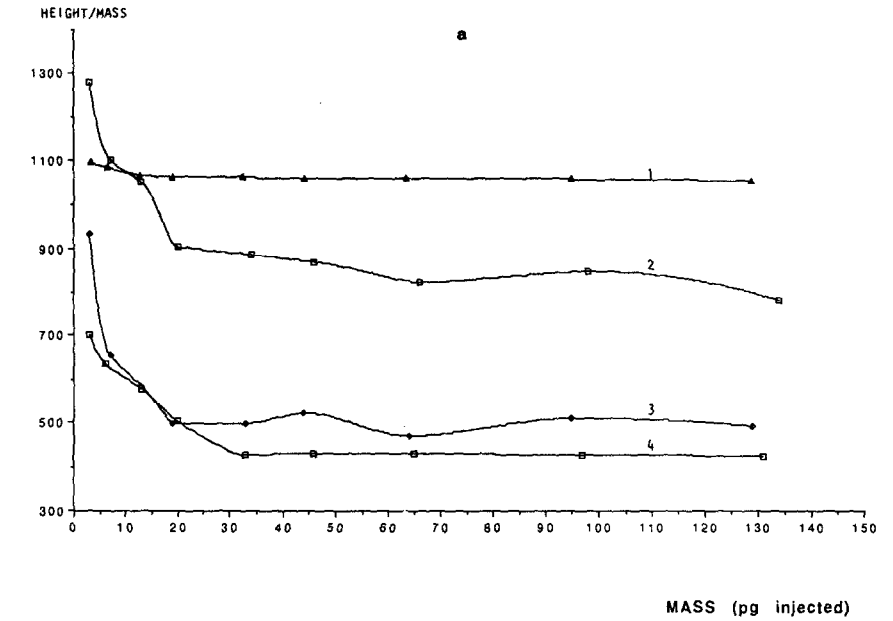
3. Results and discussion

3.1. Linear range

It is well known that the response of detectors used in gas chromatography is non-linear over the whole range of concentration. The limitations of the linear range when using ECD are well known [1,2], and most workers have attempted to calibrate the detector using the most linear portion of the response curve. In order to establish the non-linear range in each instance, several standard solutions were injected into the GC column. The response obtained, height or area counts per unit mass injected, was plotted against the mass injected in each instance. Fig. 1 shows the curves obtained. From these curves, the lower limits of linear range were obtained for each compound. It can be seen that in all instances the ECD response at very low concentrations is non-linear. This behaviour is especially important in the determination of organochlorine compounds in which the concentration level is very low and the lower and upper limits of this linear range can be very critical [3–6]. This linear range can vary according to the state of the detector, clean-up, make-up gas used [7,8], gas flow-rate, etc., and it also depends on the detector design. The presence of such a non-linear range is one of the major limitations of the standard addition procedure.

The standard addition procedure is widely applied in atomic absorption and emission spectrometry and has also found application in electrochemical analysis and other areas [9,10]. Equal volumes of the sample solution are taken, all but one are separately spiked with known, different amounts of the analyte and all are then diluted to the same volume. The instrument signals are then determined for all these solutions and the results plotted as shown in Fig. 2. As usual, the signal is plotted on the ordinate; in this instance the abscissa is graduated in terms of the amounts of analyte added (either as an absolute mass or as a concentration). The unweighted regression line is calculated in the normal way, but space is provided for it to be extrapolated back to the point on the abscissa at which $y = 0$. It is clear that this negative intercept on the abscissa corresponds to the amount of analyte in the test sample.

However, if the detector response is non-linear over the whole concentration range, such extrapolation can produce an erroneous value. This is especially true when organochlorine com-



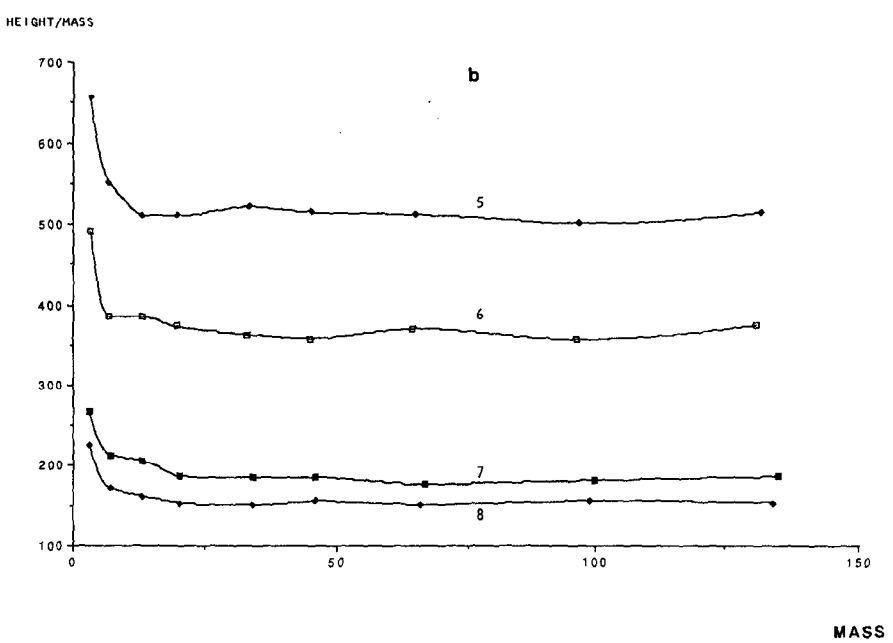


Fig. 1.

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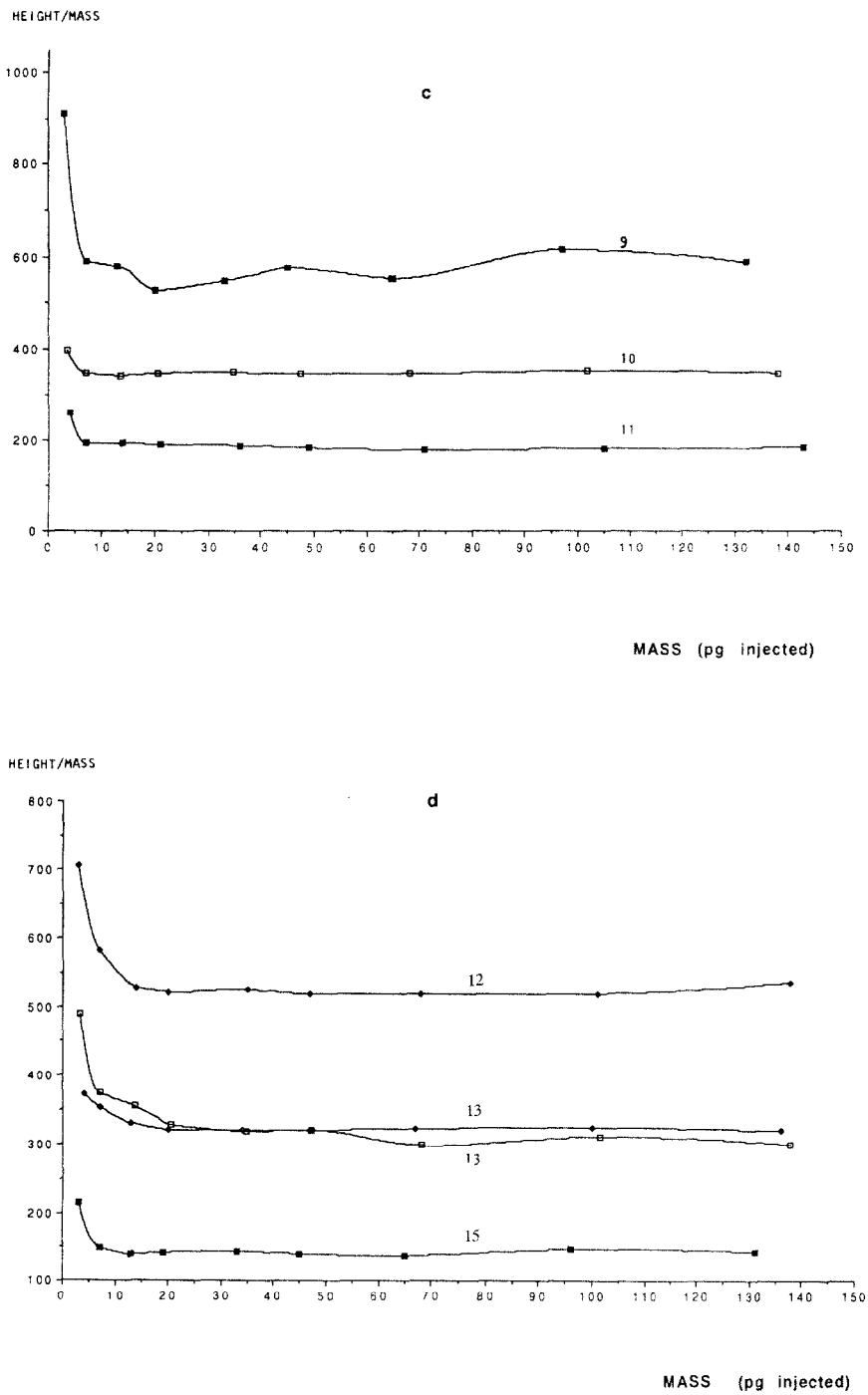

Fig. 1. ECD response *versus* the mass injected into the column. Curves: 1 = α-HCH; 2 = HCB; 3 = heptachlor; 4 = γ-chlordane; 5 = aldrin; 6 = α-endosulfan; 7 = endrin; 8 = *o,p'*-DDT; 9 = γ-HCH; 10 = dieldrin; 11 = *p,p'*-TDE; 12 = heptachlor epoxide; 13 = *p,p'*-DDE; 14 = β-HCH; 15 = *p,p'*-DDT.

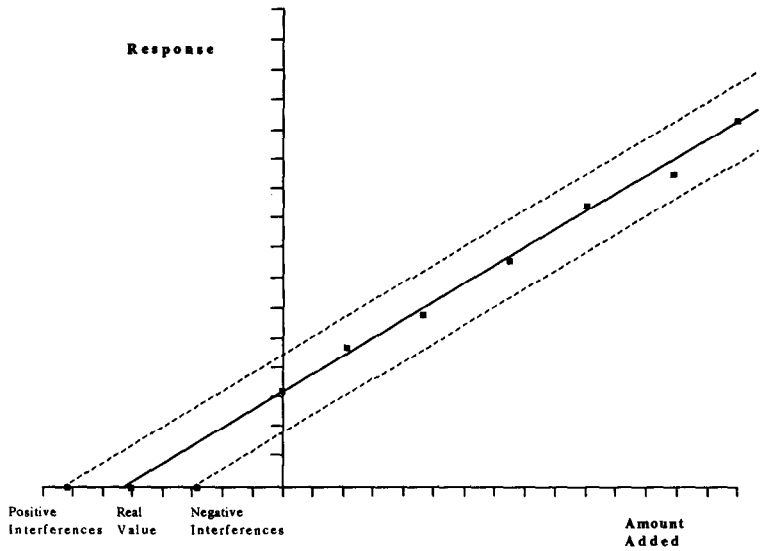

Fig. 2. Plot of a general standard addition procedure (—) and influence of matrix interferences in the standard addition procedure applied to GC–ECD (---).

pounds in real samples are determined by GC–ECD because of their low concentrations.

In order to check this theory, the standard addition procedure was applied to the determination of a series of organochlorine compounds in the certified reference material BCR CRN 115. The sample was simultaneously analysed by the normal procedure, using a calibration plot and an internal standard. The results obtained, together with the certified values and the lower limits of the linear range of the detector, are given in Table 1. It can be seen that the values

Table 1
Determination of organochlorine pesticides in BCR CRM 115 using different procedures

Compound	Lower linearity limit (ng/g)	Observed values (ng/g)	Certified values (ng/g)	Standard addition results (ng/g)[b]
α-HCH	19.15	22.26	18.47[a]	9.46 ± 2.66
β-HCH	20.50	17.74	24.20[a]	26.02 ± 1.49
γ-HCH	6.67	21.90	19.53[a]	24.56 ± 1.44
HCB	19.88	23.60	17.01	23.65 ± 8.30
Aldrin	13.01	17.36	17.42	11.92 ± 0.62
Heptachlor epoxide	6.95	68.33	17.63	7.68 ± 1.46
γ-Chlordane	33.01	55.34	53.00	48.16 ± 9.39
α-Endosulfan	6.61	48.12	44.57	45.23 ± 7.09
p,p'-DDE	20.23	53.70	47.99	51.39 ± 0.69
Dieldrin	6.98	22.10	19.00	22.06 ± 2.14
Endrin	20.10	56.96	47.92	55.31 ± 33.07
p,p'-TDE	7.24	68.29	62.94	53.14 ± 12.45
o,p'-DDT	19.98	44.15	47.40	55.92 ± 2.37

[a] Values given as indicative values in CRM report.
[b] Mean ± S.D. × *f*, where *f* is the multiplication factor according to the statistical requirements.

for α-HCH, aldrin and heptachlor epoxide obtained with the standard addition procedure differ from those obtained with the direct procedure and also from the certified values. This error could be attributed to the fact that the final concentration of the compound is near the lower limit of the linear range.

The extrapolation to zero implies that the range included is linear. When this is not the case, the standard addition procedure cannot be recommended for quantitative analysis.

It could be argued whether a larger amount of original sample could have been taken in order to obtain a more concentrated final extract containing the compounds. In such a case, the mass injected into the chromatographic column would be higher than the lower limit of the linear range. This is true when the direct interpolation procedure is applied, but with the standard addition procedure this approach only serves to move the working range to an upper part of the same straight line which has the same slope and obviously the same problems with extrapolation to zero. This effect is shown in Fig. 2.

The concentration effect is only valid when directed analysis through a calibration plot is used, and it does not work with the standard addition procedure.

3.2. Matrix effect

One of the major advantages of the standard addition procedure is that it avoids the matrix interference effects. However, this is only acceptable when the technique to which the standard addition procedure is applied is a relative technique. GC is an absolute technique, which means that the detector gives the total response to all the mass injected into the column instead of the response to the relative concentration (mass per unit volume). If a different compound, an interferent, is co-eluted with the analyte, the detector gives the total response of the sum of both compounds. Under these conditions, when the standard addition procedure is applied, the presence of positive or negative interferents affects the position of the straight line but the slope is not affected. Only a parallel line is obtained. This can be seen in Fig. 2. In such a case, the extrapolation to zero gives very different values, only one being the true value.

Consequently, the standard addition procedure does not avoid matrix interference effects, as can be established, for example, with spectroscopic methods.

3.3. Accuracy

In the analysis of real samples by GC, small or no significant differences are obtained between several independent aliquots of the sample. On applying the standard addition procedure these differences result in little variation of the slope of the straight line. Nevertheless, the extrapolation to zero amplifies the differences, and erroneous values could be obtained. The relative standard deviations are higher than those obtained by the direct interpolation procedure through a calibration plot applied to the same compounds in the analysis of the same sample, as can be seen in Table 1. From a statistical point of view, extrapolation methods are always less precise than interpolation techniques [4].

3.4. Recovery experiments

The addition of increasing and known amounts of a standard solution to a sample can be used to calculate the recovery of the analytes through the whole process, including extraction, clean-up, concentration and final analysis.

In this case, the final values have to be obtained by direct analysis using the calibration plot. When the obtained concentration values (quantified concentration) are plotted against the concentration added to the sample, a linear plot results. The slope of this straight line represents the recovery of each compound. This can be seen in Fig. 3.

Often this procedure is erroneously named "standard addition", but the quantification is achieved from a normal calibration plot using calibration solutions. In this instance, no extrapolation is used and the procedure is not affected by the aforementioned problems.

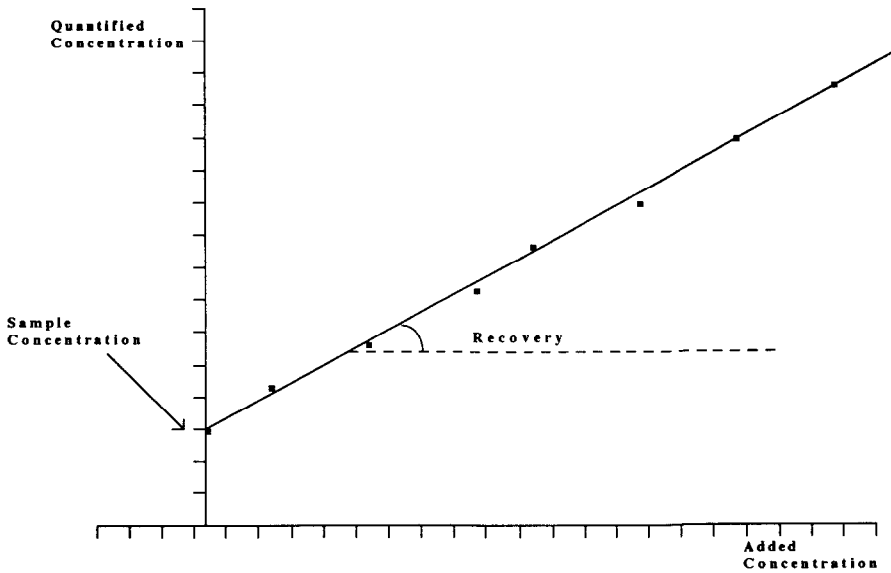

Fig. 3. Plot of quantified concentration *versus* added concentration of each compound.

4. Conclusions

The standard addition procedure cannot be applied in GC–ECD to samples containing very low concentrations of compounds that have a non-linear detector response when the final value is very near the lower limit of this linear range. The standard addition procedure does not eliminate the matrix effects in GC.

The relative standard deviations obtained by the application of standard addition procedure are higher than those obtained using the normal procedure of direct analysis using a calibration plot with calibrating solutions. When the final quantification of the samples after adding the standard is achieved by direct analysis with a normal calibration plot, the data obtained can be used to obtain the recovery of the compound.

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