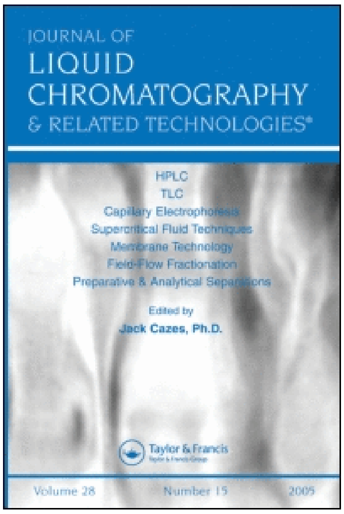

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CALIBRATION IN QUANTITATIVE TLC BASED ON WEIGHTED REGRESSION FUNCTIONS

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ABSTRACT

Two methods, one on the basis of relative error in y_i (in that case w_i should be taken as $1/y_i^2$) (YWLS) and another on the basis of $1/x_i^2$ as a weighting factor (XWLS), for the calculation of calibration function are reported and compared with conventional ordinary (OLS) and simple weighted least squares method (WLS). By these procedures the calibration functions for the TLC determination of some triazine herbicides are represented more strongly than by the ordinary least squares method mainly in the cases of heteroscedasticity. Instead of the coefficient of correlation, six coefficients of quality are used to characterize the quality of the calibration functions.

INTRODUCTION

Quantitative determinations involving instrumentation are often accomplished by establishing a calibration function. Readings from the particular instrument are plotted against known concentrations of standards and the concentration of the analyte in the sample evaluated by the underlying relationship of the calibration function.

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For quantitative analysis in the case of homoscedasticity the application of normal linear least squares method often produces an unbiased calibration function. In spite of the fact that least squares is not optimal, there is an justification for using it in the cases where the conditions are only approximately met. In particular, the Gauss - Markov theorem states that, if the errors are random and uncorrelated, the method of least squares gives the best linear unbiased estimate of the parameters; meaning that of all functions for each parameter is a linear function of the data points, least squares is one for which the variance of the parameters are smallest.[1]

If we assume only errors in the dependent variables y_i and we have a set of p parameters θ in the function $y_i = f(x_i,\theta)$, the least squares estimates of the parameters θ are those that satisfy the minimum value of the sum of squares, $ss(\theta)$, of the weighted residuals calculated with i data points.

$$ss(\theta) = \sum_i w_i (y_i - f(x_i,\theta))^2 \qquad (1)$$

w_i are the weighting factors, which are

$$w_i = 1/\sigma_i^2 \qquad (2)$$

where σ_i is the uncertainty in y_i.

In many cases no statistical information about the uncertainty of y_i is available, and in that case one should make an assumption about the error structure in the y_i values. Most often the relative error in y_i is supposed to be constant and in that case w_i should be taken as $1/y_i^2$; this is equivalent to minimizing the relative residuals:

$$ss(\theta) = \sum_i \left(\frac{y_i - f(x_i,\theta)}{y_i} \right)^2 \qquad (3)$$

When the absolute errors in y_i can be assumed constant, w_i in equation (1) can be taken as 1 for all data points.

Considering the pertinent observation about the pattern of a possible variance function, that is the variance of y is proportional to x^2, some authors[2-6] have addressed the question of whether the inverse of the x_i^2 could not be a weighting factor "with similar characteristics of weighting" like the inverse of the variance. Taking this statement into account, we have to replace w_i with $1/x_i^2$ in equation (1) to obtain the expression of slope and intercept.

In this paper the weighted least squares method using $1/y_i^2$ (YWLS) and $1/x_i^2$ (XWLS) as a weighting factor in linear regression analysis is discussed and compared with conventional ordinary (OLS) and simple weighted least squares method (WLS) in quantitative TLC. Six coefficients of quality were used to characterize the goodness of fit of the calibration functions.

EXPERIMENTAL

Chemicals and Standard Solutions

Silica gel for column chromatography was purchased from Riedle-de-Haen (Hanover, Germany), octadecyl-trichlorosilane for derivatization from Merck (Darmstadt, Germany). All solvents (methanol, dichloromethane, ethyl acetate, toluene, and acetone) were of analytical grade and were from "Chimopar" (Bucharest, Romania). Silica gel plates with fluorescent indicator and layer width of 0.5 mm were used (Sil G-50 UV254 from Macherey-Nagel, Germany). A stock standard solution of atrazine, propazine, and simazine (Merck) (0.1000 mg mL^{-1}) was prepared in methanol.

Solid Phase Extraction of the Triazine Herbicides

The solid phase, C_{18} modified silica gel, was prepared by metilation of octadecyl-trichlorosilane followed by the silanization of the silica gel. A polyethylene microcolumn (D_i = 10 mm, L = 50 mm) was filled with 1 cm^3 C_{18} modified silica gel. The column conditioning was performed by successive passing of 5 mL methanol, dichloromethane, ethyl acetate, and finally, water.

Standard solutions were processed by solid phase: 1 mL of stock solution was diluted to 1 L with distilled water and the obtained solution was passed through the extraction cartridge with a flow rate of 10 mL min^{-1}. The retained component was removed from the column with 3 mL methanol and then dried at a temperature lower than 30°C. The residue was dissolved in 1 mL methanol and this was the sample solution.

Thin-Layer Chromatography

Standard and sample solutions were applied to the preadsorbent using a variable-volume applicator (Desaga, Germany), and the plates were developed with toluene-acetone (85:15, v/v) in a normal TLC development chamber; the development distance was 50 mm. The separated triazine herbicides zones were detected by inspection under 254 nm UV light in a viewing cabinet and scanned using Shimadzu CS-9000 dual-wavelength flying spot densitometer, reflectance mode at 225 nm.

Table 1

Regression Parameters for the Calibration Data of Some Triazine Herbicides

Method	Parameter	Atrazine	Propazine	Simazine
OLS	a_0	-8465	1584	4172
	a_1	55	60	30
	s_0	6110	1812	2032
WLS	a_0	-1523	1539	3141
	a_1	37	62	31
	s_0	8087	2059	2168
XWLS and YWLS	a_0	-3943	2114	4009
	a_1	44	68	29
	s_0	6685	2168	2086

The calibration curves were determined by scanning zones via peak area, from 1 to 10 µL of TLC standard (containing 100 - 1000 ng of atrazine, propazine, and simazine) and calculating the slope, intercept, residual standard deviation, and quality coefficients (QC) using a personal linear regression program on an IBM personal computer.

Quantification was achieved by chromatographing duplicate, with three readings, 8 µL aliquots of TLC standard solution and the sample solution of atrazine and simazine and 5 µL for propazine, respectively. The sample solution was obtained by passing 1 mL from each herbicide standard solution through the extraction cartridge.

RESULTS AND DISCUSSION

The regression results concerning the intercept, a_o, slope, a_1, and the residual standard deviation, s_o, are presented in Table 1. By examining these results it appears, that from the standpoint of residual standard deviation, the best method is OLS but for atrazine and simazine best values were obtained using XWLS and YWLS compared with WLS. Also, we have to remark that the same regression results for XWLS and YWLS.

To evaluate the linearity of the methods studied, it is a good opportunity to compare the different quality coefficients (QC) used in the analytical literature to judge the goodness of fit of the regression line. In this order we present, in Table 2, the values obtained for QC_1 (4), defined as[7]:

Table 2

The Goodness of Fit of the Methods Studies in this Paper Appreciated by the Quality Coefficient QC_1, QC_2, QC_3, and QC_4, Respectively*

Method	Quality Coefficient	Atrazine	Propazine	Simazine
OLS	QC_1	0.5405	0.1383	0.0951
	QC_2	1.3890	0.2465	0.0986
	QC_3	0.2482	0.0519	0.0908
	QC_4	0.2537	0.0517	0.0910
WLS	QC_1	0.2722	0.1397	0.1019
	QC_2	1.0750	0.2568	0.0954
	QC_3	0.3874	0.0573	0.1003
	QC_4	0.3358	0.0587	0.0971
XWLS and YWLS	QC_1	0.2391	0.1893	0.0974
	QC_2	1.2040	0.3579	0.0913
	QC_3	0.2948	0.1407	0.0951
	QC_4	0.2776	0.1599	0.0935

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* See the text.

$$QC_1 = 100x\sqrt{\frac{\sum_{i=1}^{N}\left(\frac{\hat{y}_i - y_i}{\hat{y}_i}\right)^2}{N-1}}\ \% \qquad (4)$$

where y_i and $\hat{y}_i$ are the responses measured at each datum and those predicted by the model in Table 1, respectively, and N is the number of all data points, QC_2 used when measured, y_i, replace estimates $\hat{y}_i$ at the denomitor[8], and also QC_3 and QC_4, respectively, referring to the mean signal $\bar{y}$[9], instead of the signal itself and the mean of estimated signal $\hat{\bar{y}}_i$, respectively. The smaller the QC is, the better the fit of the model. Taking into account the contradictory values of QC_1- QC_4 in Table 2, we have also computed two new quality coefficients, QC_5 and QC_6, respectively, proposed and discussed in.[5,6] The first coefficient, QC_5, refers to the maximum of absolute residuals,

$$QC_5 = \sqrt{\sum_{i=1}^{N}(r_i / \max|r_i|)^2} \qquad (5)$$

Table 3

The Goodness of Fit of the Methods Studied in this Paper Appreciated by the Quality Coefficient NQC_5 and NQC_6 Respectively*

Method	Quality Coefficient	Atrazine	Propazine	Simazine
OLS	NQC_5	0.0586	0.3331	0.2691
	NQC_6	0.0986	0.0352	0.0288
WLS	NQC_5	0.2121	0.2263	0.2350
	NQC_6	0.1043	0.0566	0.0435
XWLS and YWLS	NQC_5	0.1137	0.3471	0.2552
	NQC_6	0.1108	0.0237	0.0341

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* See the text.

and QC_6 refers to the mean of the absolute residuals,

$$QC_6 = \sqrt{\sum_{i=1}^{N} (r_i / \bar{r})^2} \qquad (6)$$

It is very easy to prove that the possible values for QC_5 lie in the range $[1, N^{1/2}]$, and the possible values for QC_6 lie in the range $[N^{1/2}, N]$. So, it will be more practical to compare the values of the normalized QC_5 and QC_6 namely NQC_5 (7) and NQC_6 (8), which take values within the range [0,1]:

$$NQC_5 = \frac{QC_5 - 1}{\sqrt{N} - 1} \qquad (7)$$

and

$$NQC_6 = \frac{QC_6 - \sqrt{N}}{N - \sqrt{N}} \qquad (8)$$

By referring to the results for the quality coefficients presented in Table 3 it is easy to notice that the weighted methods are the best. WLS is the best in the case of propazine and simazine, XWLS and YWLS appear to be the best in the case of atrazine.

Table 4

The Concentration of the Two Standard Samples*

	Estimated Mean Concentration (ng/Spot) and Recovery Rate (%)								
	Atrazine			Propazine			Simazine		
Method	**b**	**a**	**Rr**	**b**	**a**	**Rr**	**b**	**a**	**Rr**
OLS	649	600	92.45	533	500	93.81	773	742	95.98
WSL	778	704	91.26	517	485	93.81	805	771	95.78
XWLS and YWLS	709	647	90.49	525	496	94.47	781	752	96.29

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* Before (b) and after passing through the extraction cartridge (a). Estimated using the regression functions in Table 1 and the recovery rate, Rr, calculated as a/b x 100.

Table 5

Results Obtained Using the Regression Methods in Table 1*

	Mean Estimated ± SD**		
Method	**Atrazine**	**Propazine**	**Simazine**
OLS	649 ± 112	533 ± 30	773 ± 68
WLS	709 ± 219	517 ± 33	805 ± 75
XWLS and YWLS	778 ± 152	525 ± 82	781 ± 67

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* Concerning the accuracy and precision for a standard sample of 800 ng for atrazine and simazine and 500 ng for propazine. ** SD = s_0/a_1 (see ref. 10).

In addition, for a more realistic comparison of the four regression functions presented in Table 1, we calculated the estimates of standard sample concentration applied on the same plate before and after passing through the extraction cartridge. The concentration of the two samples represented 800 ng per spot for atrazine and simazine and 500 ng per spot for propazine, respectively. A careful examination of results presented in Table 4 and 5 illustrates that the performances, from the accuracy and precision point of view, of weighted least squares methods exceeds that of OLS. The highest accuracy was obtained using the WLS method; the OLS appears to be more precise but it is very biased.

CONCLUSIONS

The weighted least squares methods (XWLS), (YWLS), and well known WLS, compared in this paper, use the inverse of squares of y_i, $(1/y_i^2)$, and x_i, $(1/x_i^2)$ and also the inverse of variance, $1/\sigma_i^2$, as a weighting factor in linear regression analysis. Considering the results obtained concerning the determination of some triazine herbicides it seems that the weighted least squares methods are more suitable in quantitative TLC. The similar performances (equal in the case of XWLS and YWLS) for the weighted methods could be explained considering the large number of regression points (60); duplicates with three readings for ten concentrations.

It is also important to observe that the new quality coefficients applied in this paper allow us to do a more realistic analysis of the linearity of calibration lines.

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