



ELSEVIER

Journal of Chromatography A, 750 (1996) 341–349

JOURNAL OF
CHROMATOGRAPHY A

Quantification of two chromatographic unresolved dichlorophenols using gas chromatography–direct deposition–Fourier transform infrared spectrometry and multivariate calibration

I. Rodríguez, M.H. Bollaín, R. Cela*

Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Química, Universidad de Santiago de Compostela, Compostela 15706, Spain

Abstract

A method for the quantification of two chromatographically unresolved dichlorophenol isomers in water is described. Acetylation and concentration on graphitized carbon cartridges are carried out as a preliminary step. Detection is made by gas chromatography (GC) coupled to Fourier transform infrared spectroscopy (FTIR), using a direct deposition interface (DD). Infrared spectra in the maximum of the unresolved peak of 2,5- and 2,4-dichlorophenol for a series of standards with different amounts of these two compounds are used, to elaborate a multivariate calibration model (PLS-1 algorithm). By the method described, concentrations of dichlorophenol isomers in water at ng/ml level can be determined.

Keywords: Water analysis; Environmental analysis; Multivariate calibration; Dichlorophenols; Chlorophenols; Phenols

1. Introduction

Chlorophenols are pollutants present in aqueous media due to the degradation of pesticides and insecticides [1] and to unchlorinated phenols present in water that is submitted to a chlorination process.

The US Environmental Protection Agency (EPA) [2], has compiled a list of eleven phenols considered as priority pollutants in aquatic media. Also, the European Community (EC) legislation has issued its own list of priority pollutants, including several chlorophenols [3].

Since the maximum allowable concentration of phenols in tap water is 0.5 ng/ml, their analysis usually requires a preliminary sample concentration step. Several techniques including extraction with organic solvents [4,5], solid phase extraction with

C₁₈ [6,7] and graphitized carbon cartridges [8–10] have been developed for this purpose.

Gas chromatography is the most widely used technique for the determination of phenols. However, free phenols produce broad, tailed peaks due to their high polarity, so chromatographic separation is not easy [11–13]. To avoid this problem, phenols are derivatized to less polar compounds with better chromatographic properties. Acetylation is one of the most frequently used procedures for this purpose [14].

When large volumes of water (1 l or more) are processed the chromatograms obtained using gas chromatography–flame ionization detection (GC–FID) show so many interfering peaks that it is nearly impossible to accurately identify chlorophenols. Hyphenated techniques, eg. gas chromatography–microwave-induced plasma atomic emission detection (GC–MIP–AED), enable highly selective chromatograms to be recorded, when the chlorine emission

*Corresponding author.

line is monitored [10]. However, even with this technique it is difficult to differentiate among isomeric chlorophenols with very close retention times. Neither can coupling gas chromatography to mass spectrometry (GC–MS) differentiate between overlapping structural isomers [15].

A combination of gas chromatography–Fourier transform infrared spectrometry (GC–FTIR) solves this problem using an IR spectrum for each peak, to confirm its identity. Nevertheless, applications of this technique in the environmental field have been limited, mainly due to its lack of sensitivity [16,17].

The sensitivity of the GC–FTIR coupling mainly depends on the existence in the molecules of strongly absorbing groups and on the interface used. The use of the direct deposition interface (DD) allows detection limits that are two orders of magnitude better than interfaces based on the flow-cell design (light pipe interfaces). In the GC–DD-FTIR systems, analytes eluted from the chromatographic column are deposited on a moving ZnSe slide (which is transparent to infrared radiation), located in a vacuum chamber and cooled by liquid nitrogen [18–21].

The advantages of this technique have been reported to quantify seventeen isomeric polychlorophenols (including all those considered in EPA and EC lists), allowing their accurate differentiation and quantification by means of the corresponding IR spectra [22]. Only 2,4- and 2,5-dichlorophenol, which cannot be chromatographically resolved, could not be accurately quantified. Far from being a minor problem, the analysis of 2,4- and 2,5-dichlorophenol implies a difficult task when analyzing chlorophenols in real samples. Attempts to resolve these two peaks (eg. by changing the stationary phase) lead to poor separation of the other chlorophenols that have to be monitored. On the other hand, 2,4-dichlorophenol (2,4-DCP) is suspected to be a potent carcinogenic agent [23] and is therefore included in the priority pollutant lists, while 2,5-dichlorophenol (2,5-DCP), which is much less toxic, is not included. Therefore, the risk exists of overestimating the concentration of 2,4-DCP due to the presence of 2,5-DCP, when analysing real water samples that can contain both compounds in variable proportions.

A possible alternative would be to accept that 2,4-DCP and 2,5-DCP remain chromatographically unresolved and build up a multivariate calibration

model (using a PLS-1 algorithm [24]) from the IR spectra of standard mixtures of the two chlorophenols previously derivatized to acetates. This model can then be used to predict concentrations of 2,4- and 2,5-DCP in water samples containing different amounts of the two compounds. The results presented here demonstrate the usefulness and applicability of this type of alternative procedure.

2. Experimental

2.1. Reagents

Methanol (HPLC grade), hexane (pesticide grade), potassium carbonate and acetic anhydride (99% purity) were supplied by Merck (Darmstadt, Germany). Tetramethylammonium hydroxide (TMAOH) was supplied by Aldrich (Milwaukee, WI, USA).

The standards 2,6-dichlorophenol (2,6-DCP), 2,5-DCP, 2,4-DCP, 3,5-dichlorophenol (3,5-DCP), 2,3-dichlorophenol (2,3-DCP) and 3,4-dichlorophenol (3,4-DCP) were supplied by Aldrich.

Stock solutions (4.0 mg/ml) were prepared for each standard in methanol. These solutions were stored at 4°C and were protected from light. Working solutions were prepared from the individual stocks by mixing and diluting as appropriate.

Water samples were preconcentrated by means of graphitized carbon black cartridges, Supelclean, ENVI-Carb SPE (Supelco, Bellefonte, PA, USA) of 0.25 g.

2.2. Equipment

A Hewlett-Packard (HP) (Avondale, PA, USA) model 5890 series II plus gas chromatograph, equipped with electronic pressure control (EPC) and an on-column injector was connected to the FTIR instrument. The chromatographic column was a BP-5 (Scientific Glass Engineering, Ringwood, Victoria, Australia) (30 m×0.32 mm I.D.) with methylphenylsilicone (0.25 μm film thickness). Helium (99.999%) was used as the carrier gas. Optimal parameters have been summarized in Table 1.

Gram-Schmidt reconstructed chromatograms (GSC), functional group chromatograms (FGC) and

Table 1
GC–DD-FTIR conditions in the separation of chlorophenols (as acetates)

Chromatographic parameters	
Injection port	On column
Injection port temperature	250°C
Injection volume	1.5 μ l
Column flow	0.9 ml/min
<i>Oven Program</i>	
Initial temperature	60°C
Initial time	1 min
Rate	15°C/min
Temperature	115°C
Time	5 min
Rate	3°C/min
Temperature	175°C
Rate	30°C
Temperature	250°C
Final Time	10 min
<i>Tracer conditions</i>	
Transfer line temperature	250°C
Tip temperature	250°C

IR spectra were recorded using a Bio-Rad Digilab (Cambridge, MA, USA), FTS 45A spectrometer equipped with a Digilab Tracer direct deposition interface and a MCT (Mercury–cadmium telluride) detector that is able to record IR spectra between 4000 and 700 cm^{-1} . The spectroscopic system is controlled by a Bio-Rad SPC 3200 data station. Spectra were collected by averaging four scans at a resolution of 8 cm^{-1} . Multivariate calibration models were built using the PLS-plus software for GRAMS/386 from Galactic Industries (Salem, NH, USA).

2.3. Acetylation of standards

The procedure followed for the acetylation of phenol standards, used for calibration or for the library is based on that proposed by Renberg and Lindstrom [25] and recently optimized by us [26]. A 2-ml volume of a 5% K_2CO_3 solution and 2 ml of hexane containing 200 μ l of acetic anhydride are added to 1 ml of a methanolic solution with different concentrations of the studied phenols in the range of 0.1–5 $\mu\text{g}/\text{ml}$. The mixture is stirred for 1 min and the organic phase is separated. Extraction is repeated with 1 ml of hexane (now without derivatizing

agent). Both hexane phases are combined, the extract is dried with anhydrous sodium sulphate and it is then injected on the chromatographic column.

2.4. Sample treatment

A known amount of a standard of chlorophenols in methanol is added (in the case of spiked samples) to the sample of water (Milli-Q or tap water, previously filtered using a 0.22- μm pore size aqueous membrane) and pH is adjusted to 11–11.5 with K_2CO_3 . Then acetic anhydride is added (5 ml per litre of sample) and the mixture is submitted to mechanic stirring for 15 min [27,28]. Finally, a small volume of methanol (ca. 2% of the volume of the sample) is added and the spiked water sample is loaded on a previously conditioned carbon cartridge.

The conditioning of the cartridge is carried out as follows: (a) Washing with 5 ml of methanol and (b) activation of the cartridge with 5 ml of Milli-Q water at pH 2–3.

After sample loading, the cartridges are dried for 20 min using a N_2 stream and the chlorophenols are eluted with 3 ml of hexane containing 1% TMAOH (with the current in the opposite direction to that of sample flow). The extract is concentrated by a N_2 stream at room temperature to a final volume of 0.5 ml (using a Turbo Vap II Workstation, Zymark, Hopkinton, MA, USA) and it is injected into the chromatographic system.

3. Results and discussion

Under the working conditions shown in Table 1, quantification limits ($S/N=10$) of 0.1 $\mu\text{g}/\text{ml}$ have been obtained for each of the studied dichlorophenols, when the chromatogram corresponding to the absorption in the IR region between 1750 and 1850 cm^{-1} was monitored [22]. Different oven temperature programs were employed in an attempt to separate 2,5-DCP from 2,4-DCP, but both peaks always remained fully overlapping. Even when the movement rate for the ZnSe slide in the tracer interface was increased (which also affects chromatogram resolution), it was impossible to resolve these compounds.

To confirm that bad resolution was not caused by

the slide speed, acetylated standard mixtures of the two components were also injected in a GC–MS system, using an identical column. Since both peaks also remained unresolved in this system, it is reasonable to assume that overlapping peaks are due to the characteristics of the column rather than to the interface used in the GC–FTIR hyphenation.

3.1. Qualitative analysis

Identification of the peaks corresponding to each dichlorophenol isomer was described in a previous paper [22]. Euclidean distances were used as a measure of the similarity between IR library spectra, obtained for acetylated standards of each compound, and spectra for the peaks appearing in a given chromatogram. The smaller the value of Euclidean distance, the better the identification.

In the case of the overlapping peak including 2,5-DCP and 2,4-DCP, it is very difficult to make accurate identifications using Euclidean distances, because peak spectra are the combination of IR spectra for pure 2,5- and 2,4-DCPs.

When water samples containing both compounds at similar concentrations are analysed (Table 2, Sample 1), it is possible to identify 2,5-DCP at the beginning of the peak and 2,4-DCP at the tail, while at the peak apex approximately equal values of Euclidean distances for both compounds are obtained. This suggests a 50% contribution of each compound to the global spectra at this point. As the substances eluted from the column remain trapped on the ZnSe slide, it is possible to obtain a spectrum of this peak using more scans, with a better signal-to-noise ratio, and the results confirmed previous suppositions (Table 2, Sample 1, sixteen scans).

However, when the samples contain both compounds at different concentrations (Table 2, Sample 2), the more concentrated phenol has the smaller value for the Euclidean distance at any position in the peak (beginning, maximum and tail). Therefore, the whole peak would be identified as corresponding to only one compound (the more concentrated one) and there would be an overestimation of the concentration of the main compound due to the presence of the other isomer.

3.2. Development of a multivariate calibration model

Multivariate calibration techniques have been developed to quantify mixtures of several compounds, when their analytical spectra (usually IR or UV spectra), do not show sufficiently characteristic bands at different wavelengths. In this situation, several algorithms have been reported that allow quantification of the components in the mixture using information contained in the global spectra [29,30].

Here, a multivariate calibration model (using a PLS-1 algorithm) was developed, using IR spectra recorded at 8 cm^{-1} , in the maximum of the unresolved chromatographic peak for a series of eleven acetylated standards as analytical information (Table 3). This group of standards are the training samples.

Concentrations of 2,4-DCP and 2,5-DCP in the training group of standards must be uncorrelated, otherwise the spectral decomposition algorithm cannot evaluate the contribution of each phenol to the spectra of the mixture, which in turn leads to poor model predictions. Fig. 1 shows the lack of collinearity between 2,5-DCP and 2,4-DCP concentrations in the training set standards.

Training spectra suffer two preprocessing operations before developing the model; baseline correction and mean centring. As both chlorophenols present spectral bands in the region between 1860 and 700 cm^{-1} (Fig. 2), this was, as a first approach, the spectral region chosen to develop the calibration model. Optimization was carried out using a cross-validation process, using a "leave-one-out" approach. In this process, each standard is left out of the training set, making a model with the other ten, and using this restricted model to predict the concentrations of 2,4-DCP and 2,5-DCP in the left-out standard. Differences (residual) between the real concentration of the two phenols and the concentration estimated by the model is the prediction error for this standard. This mechanism is repeated in a systematic way for the eleven standards and the PRESS (Prediction residual error sum of squares) is calculated. The smaller the PRESS, the better the multivariate calibration model.

In a system with only two components (analytical signal is the combination of the spectra for 2,4-DCP

Table 2
Identification of 2,5- and 2,4-DCPs (using Euclidean distances) in spiked water samples, using an IR library made in the laboratory

Sample number	Concentration (ng/ml)	Four scans per spectrum						Sixteen scans per spectrum							
		2,4-DCP			2,5-DCP			2,4-DCP			2,5-DCP				
		Beginning	Maxim.	Tail	Beginning	Maxim.	Tail	Beginning	Maxim.	Tail	Beginning	Maxim.	Tail		
1	0.55	0.56	0.38	0.34	0.28	0.35	0.35	0.36	0.35	0.32	0.27	0.24	0.24	0.27	0.33
2	4.54	1.66	0.21	0.19	0.19	0.30	0.25	0.27	0.30	0.22	0.17	0.15	0.27	0.30	0.31

Table 3
Concentrations of standards used as experimental samples to develop the multivariate calibration model

Standard number	2,5-Dichlorophenol ($\mu\text{g/ml}$)	2,4-Dichlorophenol ($\mu\text{g/ml}$)
1	0.63	0
2	1.46	1.42
3	0	4.25
4	0.78	2.84
5	1.46	5.67
6	2.92	0.95
7	0.49	0.47
8	0.51	0.49
9	3.88	0.95
10	0	0.95
11	0.98	1.89

and 2,5-DCP plus noise), two factors must suffice to create a model. Nevertheless, the final representation of PRESS as a function of a number of factors reaches a minimum with three factors (Fig. 3a) for both isomers. As the difference in the PRESS value from two to three factors is small, and the coefficients (scores) for the third factor are also small (Fig. 3b), it was assumed that the third factor is probably adjusting the noise of the spectra, thus producing an overfitted model. So, two factors were taken to create a model of the system with acceptable correlation coefficients between real and predicted concentrations for the calibration set (Table 4a).

3.3. Influence of the spectral region taken in the PLS-1 results

One common characteristic of 2,5-DCP and 2,4-DCP spectra is the presence of two strong bands at 1770 and 1195 cm^{-1} . These bands correspond to the

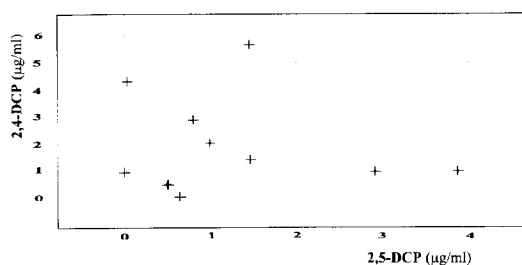


Fig. 1. Diagram of the 2,5-DCP and 2,4-DCP concentration in the experimental samples.

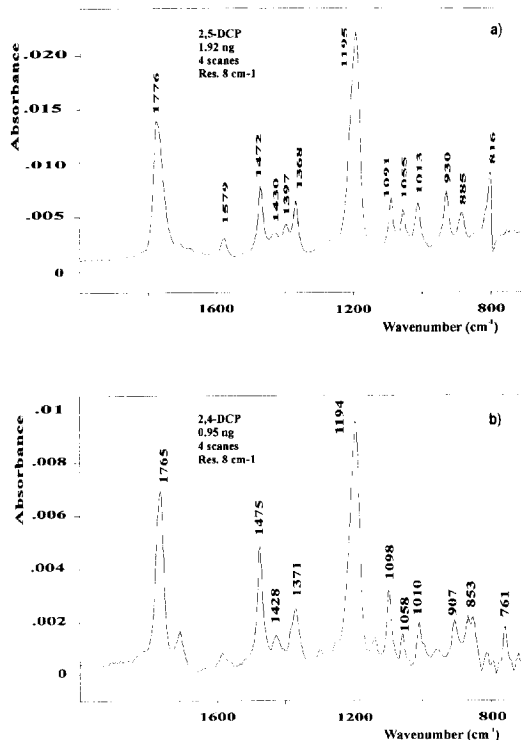


Fig. 2. IR spectra of 2,5-DCP (1.92 ng) and 2,4-DCP (0.95 ng), as acetyl derivatives, at four scans per spectrum.

vibrations of carbonyl groups in the acetylated chlorophenols and to those of the double bonds in the aromatic ring. Other minor bands, located in the regions 1500–1350 and 1000–700 cm^{-1} , and of lower intensity, allow us to differentiate between the two compounds (Fig. 2).

In fact, it was observed that the inclusion of the 1770 and 1195 cm^{-1} bands in the model decreases the efficiency of the PLS-1 predictions. Two new models were developed (Table 4a–b), using the spectral regions 1535–1323 and 1126–705 cm^{-1} (Model 2) and 1000–705 cm^{-1} (Model 3). In both cases two factors appear to provide a model of the system, with a lower standard error of cross-validation (SECV) and better correlation coefficients (R^2) than those characterized by the first model (Table 4). Consequently, it appears that the inclusion in the multivariate model of spectral regions containing common strong absorption bands for the analytes limits the efficiency of the predictions. By choosing significant spectral regions, good predictions for the

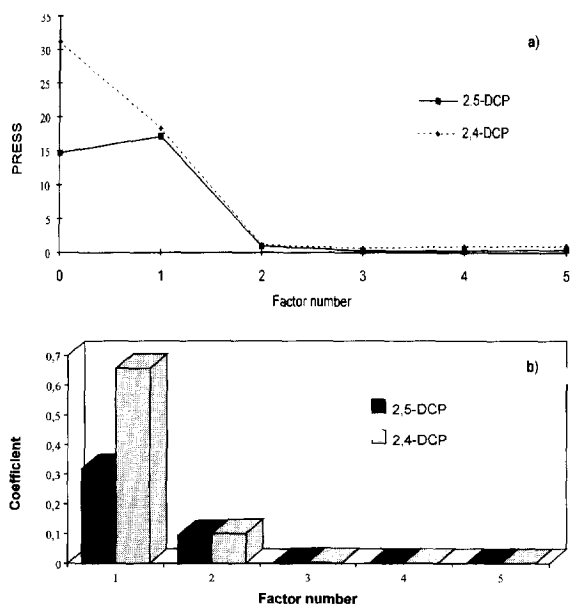


Fig. 3. (a) Variation of PRESS as a function of the factor number for 2,5-DCP and 2,4-DCP, using a PLS-1 algorithm between 1860–700 cm^{-1} ; (b) scores for factors of this model

Table 4
Results of the PLS-1 algorithm

Component	Number of factors	SECV	R^2
(a) Model 1			
Spectral region (1860–705 cm^{-1}). Total points 150			
2,5-DCP	2	0.307	0.955
2,4-DCP	2	0.336	0.966
(b) Model 2			
Spectral region (1535–1323 cm^{-1}). Total points 23			
Spectral region (1126–705 cm^{-1}). Total points 55			
2,5-DCP	2	0.132	0.991
2,4-DCP	2	0.316	0.970
(c) Model 3			
Spectral region (1000–705 cm^{-1}). Total points 19			
2,5-DCP	2	0.161	0.987
2,4-DCP	2	0.238	0.982

SECV: Standard error of calibration.

R^2 : Coefficient of correlation.

$$SECV = \sqrt{\frac{\sum_{i=1}^n (Y_{ki} - Y_{pi})^2}{n-1}}$$

Y_k : Known concentration.

Y_p : Predicted concentration.

set of calibration data can be achieved, even for a model with very few points, such as Model 3 (Fig. 4).

3.4. Application to real samples

Model 3 shows the best correlation coefficient (R^2) and the smallest SECV for 2,4-DCP, which is the compound of concern from the regulatory point of view. Therefore, this model has been used to estimate the concentration of 2,5- and 2,4-DCPs in water samples (500 ml) spiked with both isomers at different concentration levels. These samples were derivatized and concentrated according to the procedure described in Section 2.

Results obtained for each sample (mean values) and percentages of recovery as a function of spiked concentrations are summarized in Table 5. These data suggest recoveries of around 90% in the range of spiked levels.

If we compare these results with those obtained for the remaining DCP isomers (Table 6) obtained by using an identical concentration–derivatization procedure and measured with the same instrumental system, but using the conventional external standard univariate calibration (concentration versus chromatographic peak height or area, recorded in the functional region between 1860–1750 cm^{-1}), we can see that recoveries for these compounds also range around 90%. This suggests that recoveries are mainly influenced by the sample concentration–derivatiza-

Table 5
2,5-dichlorophenol and 2,4-dichlorophenol concentrations measured in samples of 500 ml of Milli-Q water spiked with these two compounds using a PLS-1 algorithm (Model 3)

Sample number	2,5-DCP concentration (ng/ml)		2,4-DCP concentration (ng/ml)	
	True	Found	True	Found
1	1.17	0.94	1.13	0.97
2	3.50	3.25	2.27	1.89
3	4.67	4.68	2.27	2.38
4	1.17	1.21	4.54	3.61
5	2.33	1.84	7.74	6.23
Compound	Mean recovery (%)		SD	
2,5-DCP	91%		11.2	
2,4-DCP	87%		10.4	

Table 6

Recoveries of chromatographically resolved dichlorophenols spiked in Milli-Q water. Results obtained using a linear calibration model (concentration versus chromatographic peak height, IR absorbance between 1860–1750 cm^{-1} was monitored)

Compound	Sample concentration (ng/ml)	Sample volume			
		1000 ml		500 ml	
		Recovery (%)	SD	Recovery (%)	SD
2,6-DCP	1.07	80	7.8	83	7.4
3,5-DCP	1.24	87	7.3	91	7.9
2,3-DCP	1.12	88	12.5	85	4.4
3,4-DCP	1.10	97	8.7	98	13.2

tion process and that the two overlapping DCPs can be measured with the same accuracy and precision as the remaining ones in just one chromatographic run, by the use of the proposed multivariate calibration procedure. This conclusion was further confirmed by measuring a spiked sample containing only 2,4-DCP,

using the same concentration–derivatization process but resorting to a GC–MIP–AED system [25]. In this case, recoveries of $86 \pm 4.5\%$ were estimated.

4. Conclusions

Application of a PLS-1 algorithm to IR spectra, obtained in the maximum of the 2,4 plus 2,5-DCP peak, allowed the development of a multivariate calibration model that can be used to quantify these two chromatographically unresolved isomers at the same level of accuracy and precision as for the remaining chromatographically resolved ones. This avoids the need to use two different columns and independent injections in the control of priority chlorophenol pollutants in water samples, while assuring that target compounds are unequivocally identified and quantitated.

Acknowledgments

This work was supported by the Spanish Inter-ministerial Commission for Science and Technology (Project PB92-0372).

References

- [1] F.B. Dewalle, D.A. Kalman, R. Dills, D. Norman, E.S.K. Chain, M. Giabbai and M. Ghosal, *Water Sci. Technol.*, 14 (1982) 143.
- [2] L.H. Keith (Editor), *Compilation of Sampling Analysis*

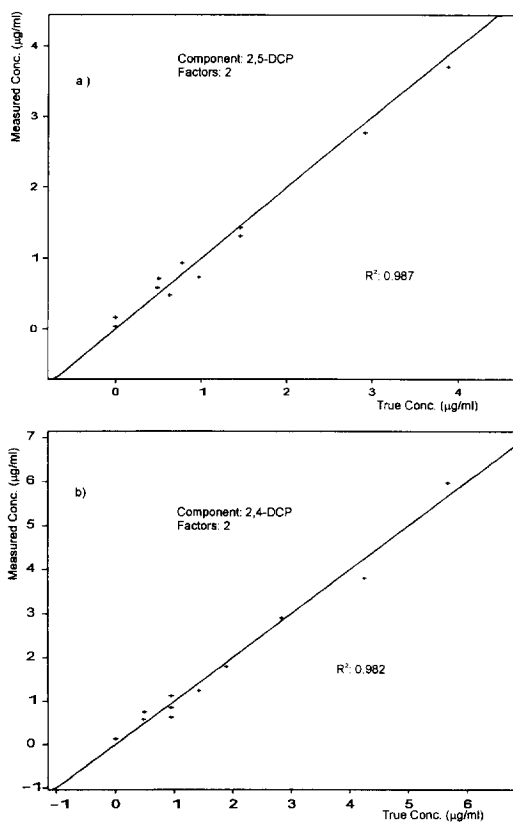


Fig. 4. Correlation between the true and measured concentrations of the calibration samples. (a) 2,5-DCP and (b) 2,4-DCP, using Model 3.

- Methods, US Environmental Protection Agency, Boca Raton, FL, 1991, pp. 389–486.
- [3] Directiva 80/77/CEE 15-7-1990; Diario Oficial de las Comunidades Europeas 30/8/1990, European Community, Brussels, 1990.
- [4] R.A. Booth and J.N. Lester, *J. Chromatogr. Sci.*, 32 (1994) 259.
- [5] N.D. Bedding, A.E. McIntyre, J.N. Lester and R. Perry, *J. Chromatogr. Sci.*, 26 (1988) 597.
- [6] A. Geibler and H.F. Scholer, *Water Res.*, 28 (1994) 2047.
- [7] S.A. Schuette, R.E. Smith, L.R. Holdan and J.A. Graham, *Anal. Chim. Acta*, 236 (1990) 141.
- [8] A. Di Corcia, S. Marchese and R. Samperi, *J. Chromatogr.*, 642 (1993) 163.
- [9] A. Di Corcia, S. Marchese, R. Samperi, G. Cecchini and L. Cirilli, *J. Assoc. Off. Anal. Chem.*, 77 (1994) 446.
- [10] M.I. Turnes, I. Rodríguez, M.C. Mejuto and R. Cela, *J. Chromatogr. A*, 683 (1994) 21.
- [11] P. Mubmamn, K. Levsen and W. Radeck, *Fresenius' Z. Anal. Chem.*, 348 (1994) 654.
- [12] T.J. Boy, *J. Chromatogr. A*, 662 (1994) 281.
- [13] J. Drozd, *Chemical Derivatization in Gas Chromatography* (Journal of Chromatography Library, Vol. 19), Elsevier, Amsterdam, 1981, pp 53–84.
- [14] H. Lee, R.L. Hong-You and A.S.Y. Chau, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 422.
- [15] V. Janda and H. Van Langenhove, *J. Chromatogr.*, 472 (1989) 327.
- [16] P. Doumenq, M. Guiliano and G. Mille, *Talanta*, 39 (1992) 149.
- [17] D.F. Gurka and S.M. Pyle, *Environ. Sci. Technol.*, 22 (1988) 963.
- [18] A.M. Haefner, K.L. Norton, P.R. Griffiths, S. Bourne and R. Curbelo, *Anal. Chem.*, 69 (1988) 2441.
- [19] R.S. Brown and C.L. Wilkins, *Anal. Chem.*, 60 (1988) 1483.
- [20] S. Bourne, A.M. Haefner, K.L. Norton and P.R. Griffiths, *Anal. Chem.*, 62 (1990) 2448.
- [21] N.R. Smyrl, D.M. Hembre, W.E. Davis, D.M. Williams and J.C. Vance, *Appl. Spectrosc.*, 46 (1992) 277.
- [22] I. Rodríguez, M.H. Bollaín, C.M. García and R. Cela, *J. Chromatogr. A*, in press.
- [23] B.K. Afghan and A.S.Y. Chau (Editors), *Analysis of Trace Organics in the Aquatic Environment*, CRC Press, Boca Raton, FL, 1991, pp. 120–126.
- [24] H. Martens and T. Naes, *Multivariate Calibration*, Wiley and Sons, Chichester, 1989.
- [25] L. Renberg and K. Linsdtrom, *J. Chromatogr.*, 214 (1981) 327.
- [26] I. Rodríguez, M.I. Turnes, M.C. Mejuto and R. Cela, *J. Chromatogr. A*, 721 (1996) 297.
- [27] R.T. Coutts, E.E. Hargesheimer and F.T. Pasutto, *J. Chromatogr.*, 179 (1979) 291.
- [28] C. Schlett and B. Pfeifer, *Vom Wasser*, 79 (1992) 65.
- [29] D.M. Haaland and E.V. Thomas, *Anal. Chem.*, 60 (1988) 1193.
- [30] D.M. Haaland, *Anal. Chem.*, 60 (1988) 1208.