



ELSEVIER

Journal of Chromatography A, 907 (2001) 81–88

JOURNAL OF
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Bi-quadratic surface response for quantitative determination of analytes leading to partially overlapped chromatographic peaks

Giuseppe Pace^{a,*}, Adriano Berton^a, Luca Bergamaschi^b

^a*Centro di Chimica e Tecnologia dei Composti Metallorganici degli Elementi di Transizione del CNR, Via Marzolo 9, 35131 Padova, Italy*

^b*Dipartimento di Metodi e Modelli Matematici per le Scienze Applicate, Università di Padova, Via Belzoni 7, 35131 Padova, Italy*

Received 25 May 2000; received in revised form 3 October 2000; accepted 9 October 2000

Abstract

A simple analytic approach is described for the accurate determination of the concentration of components which give rise to partially overlapped chromatographic peaks. This method requires neither deconvolution processes nor chromatographic separation of the peaks. From the empirical point of view a bi-quadratic function has been found relating the area of an unresolved peak with the concentrations of the related analyte and of the interfering one. Such concentrations are then computed by solving the related non linear system of equations by the Newton–Raphson method. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Bi-quadratic surface response; Partially overlapping peaks; Peak overlap

1. Introduction

Instrumental chromatography is widely employed for the separation and simultaneous quantitative assessment of a large variety of analytes, which are present in aqueous and non aqueous solutions [1–7]. Although this technique has been extensively studied, a problem to be solved concerns the separation of unresolved peaks, reflecting on the quality of quantitative measurements. The accurate evaluation of peak areas is particularly important mainly in the analysis of traces of analytes or when interferent compounds are present in large quantities [7–10]. However, even with careful choice of the stationary and the eluent phases, often the chromatograms

contain overlapped peaks. In such a case, the determination of the areas of the overlapped peaks present in the chromatogram, and therefore of the concentrations of the compounds corresponding to the peaks, turns out to be a very difficult problem.

To this end, different methods based on various mathematical approaches (Gaussian, exponential modified Gaussian, Poisson, etc.) have been proposed [11–24]. In these cases, positive results can only be attained if the entire chromatogram is available. This results in a postprocessing of the chromatographic data, and a consequent increase of the time devoted to the analysis. Furthermore they do not prove satisfying if applied to routine analysis. It must be emphasized that the accuracy of the estimated parameters of the functions rapidly decrease with the decrease of resolution; consequently the use of these procedures leads to errors on the evaluation

*Corresponding author. Tel.: +39-49-8756-967; fax: +39-49-8275-525.

of the area and/or the height of the peaks, reflecting on the concentration evaluation.

It is therefore difficult to employ a general method giving rise to valid results in all the possible different situations.

An interesting approach has been proposed by Mori [25], in which the maxima signals of the partially resolved peaks are linearly related to the sum of the signals corresponding to the single pure components. An extension of this approach is due to Lundeen and Juvet Jr. [26], by employing the linear sum not necessarily on peak maxima. However some limitations of these methods lie in the fact that the mathematical treatment of the data needs a high constancy of the elution time. This can be achieved either by an accurate flow control or by a further re-processing of the chromatographic data, reflecting in higher times and costs of analysis; consequently these approaches are not fully convenient for routine analyses or automatic control systems. Furthermore the presence of interfering compounds, even if at low concentration level, as chemical noise level, can lead to unreliable results.

To overcome these aspects, we have developed a simple approach based on the empirical relationship between concentrations and peak areas, computed by either the perpendicular at the valley or the linear baseline methods, for the determination of the concentrations of two components giving partially overlapped peaks. This approach can be successfully applied when the two components exhibit different retention times and the chromatographic resolution is enough to put in evidence an, even if just sketched, valley between the two peak maxima, independently of the peak shape.

In this paper the application of this approach in the analyses of different ion couples by ion chromatography is reported.

2. Experimental

2.1. Chromatographic analyses

Analyses of bromide, chlorate, sodium and ammonium ions were performed by ion chromatography with a Dionex Instrument DX 300 (Dionex, Sunnyvale, CA, USA). Anion analyses: column: IonPac

AS4A-SC, 250×4 mm; guard column: IonPac AG4A-SC, 50×4 mm; eluent: 1.7 mM NaHCO₃, 1.8 mM Na₂CO₃; flow-rate: 2.0 ml/min; detector: PED system, conductivity mode. Cation analyses: column: IonPac CS12, 250×4 mm; guard column: IonPac CG12, 50×4 mm; eluent: 20 mM methanesulfonic acid; flow-rate: 1.0 ml/min; detector: pulsed electrochemical detector (PED) system, conductivity mode. For instrument control and data acquisition, the integration system AI-450, provided by Dionex, has been used.

2.2. Reagents

All the chemicals used were of analytical-reagent grade. The working solutions containing both bromide and chlorate ions (both in the range 5–25 mg/l) or sodium (range 1–150 mg/l) and ammonium (1–15 mg/l) ions were prepared by appropriate dilution of 1 g/l stock solutions of the components. Standard solutions and eluents were prepared in Milli-Q water (18 MΩ cm resistivity at 25°C obtained from a Millipore deionizer and filtered on 0.2-μm cellulose filters).

3. Results and discussion

In order to obtain quantitative data from chromatographic analyses of two component mixtures (1 and 2) we need a function relating their concentration (c_1 and c_2) with the corresponding peak areas (A_1 and A_2). In the case of only one component the relationship between peak area and concentration would be linear and hence a simple first degree function would be sufficient. In the case of partially resolved peaks the concentration of the first component affects also the area of the second one and vice versa, so that a quadratic function describes more correctly such a relation.

As examples, two ion couples, chlorate/bromide and sodium/ammonium, have been considered. In the former case the chlorate and bromide anions exhibit a similar chromatographic response, leading to chromatograms shown in Fig. 1a. In the latter case, sodium and ammonium exhibit significant differences (see Fig. 1b): strongly different chro-

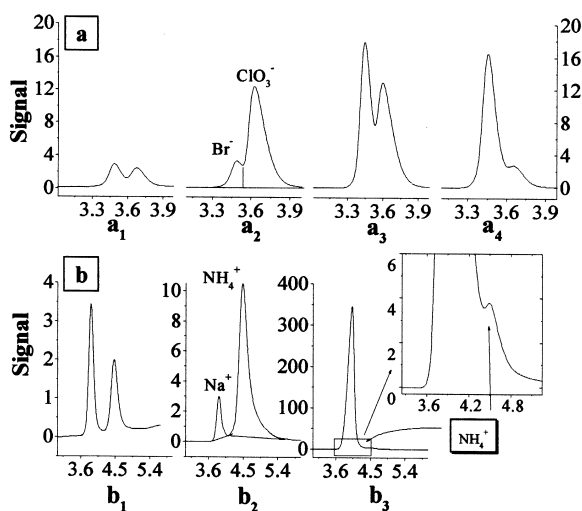


Fig. 1. (a) Coelution of bromide/chlorate anions at different concentrations, mg/l (a_1 : 5, 5; a_2 : 5, 25; a_3 : 25, 25; a_4 : 25, 5). (b) Coelution of sodium/ammonium cations at different concentrations, mg/l (b_1 : 1, 1; b_2 : 1, 15; b_3 : 150, 1).

matographic responses, due either to the different concentrations considered in the experiments or to the weak acid character of the ammonium ion which

is present in solution in dissociate and undissociate forms, are evidenced.

This aspect is well described by the analyses of solutions containing different relative concentrations of the analytes chlorate/bromide (in the range 5–25 mg/l), sodium (in the range 1–150 mg/l)/ammonium (in the range 1–15 mg/l).

The plots of Fig. 2 evidences the low influence of bromide in the area peak evaluation of chlorate ion (see Fig. 2a), and the significant interference of the sodium on the ammonium peak area evaluation (see Fig. 2b). The peak areas employed to draw Fig. 2a have been obtained by the perpendicular at the valley method, while those related to Fig. 2b have been computed by the linear baseline method. As will be discussed later, the two methods lead to practically superimposable results. In both cases, the effect of the increasing interferent ion concentration is more evident for the highest concentration value of the chlorate or ammonium ions.

Fig. 3a and b display the correlations between the area of an ion vs. its concentration for different interferent ion concentrations. In both cases the relationship area vs. concentration has been empiri-

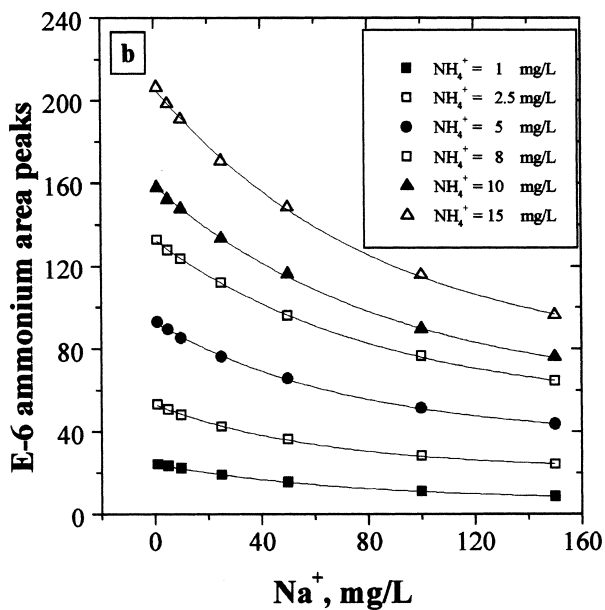
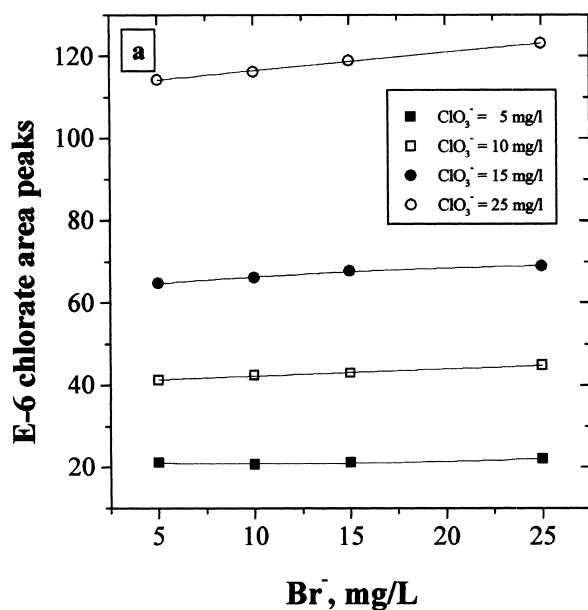


Fig. 2. Influence on the peak area of the chlorate ion (range 5–25 mg/l) (a) and ammonium ion (range 1–15 mg/l) (b) of the interferent ion (bromide and sodium, respectively) concentration.

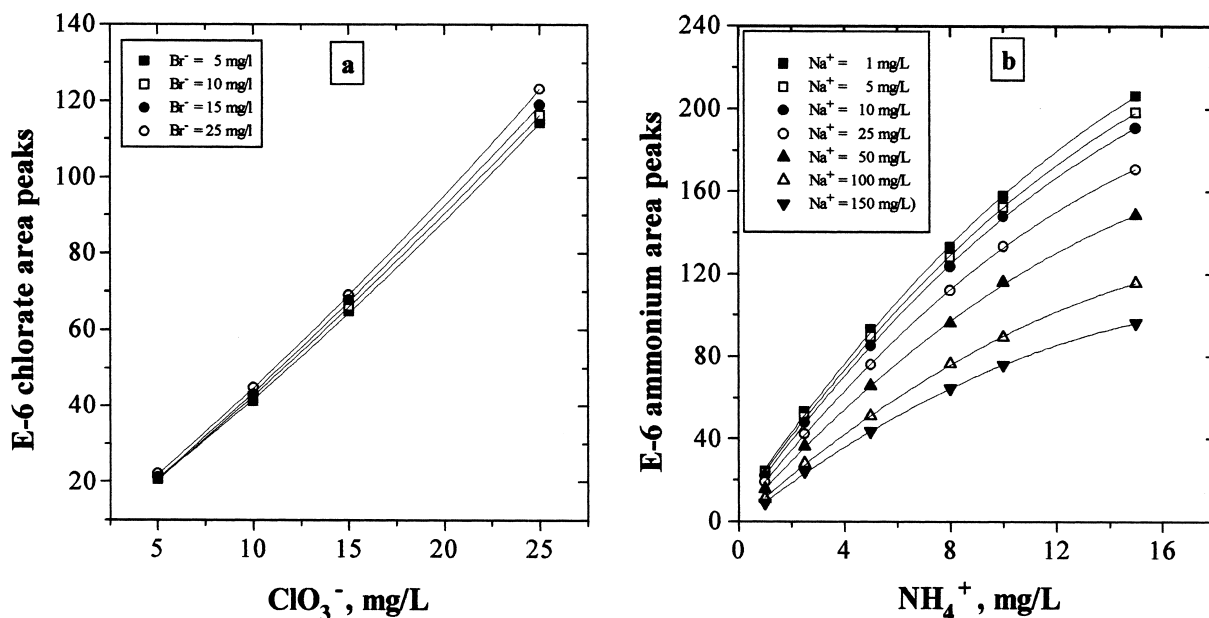


Fig. 3. Calibration plots $A_i=f(c_i)$ of chlorate (a) and ammonium (b) ions in the presence of different concentrations of the respective interferent ion (bromide, range 5–25 mg/l, and sodium, range 1–150 mg/l, respectively).

cally found to be well described by a quadratic function. The data shown in Fig. 3 indicate that different calibration plots are required, one for each value of concentration of the interferent ion.

To simplify this aspect, the approach described in this work is based on the simple correlation between the peak areas of the component of interest and the concentrations of both components leading to the partially overlapped peaks. For this aim we define a function $A_i=f(c_1, c_2)$ where the peak area of the component i (A_i) is related to both component concentrations (c_1 and c_2). The choice of the most appropriate function has been driven by two aspects: (i) the fact that there is a quadratic relationship between peak area and interferent ion concentration (see Fig. 2); (ii) the observation that the relationship between the peak area and the concentration of an ion is still a quadratic function (see Fig. 3):

$$A_1(c_1) = \alpha_0 + \alpha_1 c_1 + \alpha_2 c_1^2 \quad (1)$$

Since the area depends also on the concentration of the interferent ion (c_2) the coefficients α_0 , α_1 , α_2 of Eq. (1) will depend on c_2 . This dependence is still quadratic, as can be seen by the experimental data

reported in Fig. 4, in the case of the sodium/ammonium ions (analogous results have been achieved for chlorate/bromide ion couple):

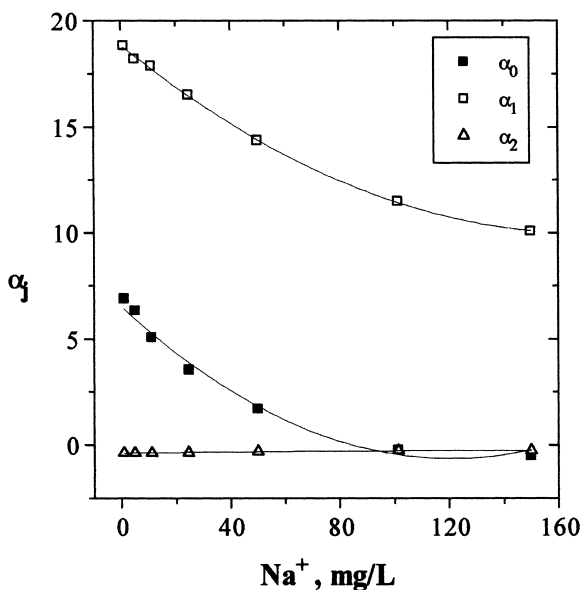


Fig. 4. Plots of α_j vs. c_2 obtained for the sodium/ammonium ion couple.

$$\alpha_j(c_2) = a_0 + a_1c_2 + a_2c_2^2 \quad (j = 0, 1, 2) \quad (2)$$

If now this expression of α_j is inserted in Eq. (1), a function in the two variables c_1, c_2 is obtained:

$$A_1(c_1, c_2) = a_{00} + a_{01}c_2 + a_{02}c_2^2 + a_{10}c_1 + a_{11}c_1c_2 + a_{12}c_1c_2^2 + a_{20}c_1^2 + a_{21}c_1^2c_2 + a_{22}c_1^2c_2^2 \quad (3)$$

where $a_{h,k}$ are the function coefficients to be calculated.

An analogous equation is obtained for A_2 :

$$A_2(c_1, c_2) = b_{00} + b_{01}c_2 + b_{02}c_2^2 + b_{10}c_1 + b_{11}c_1c_2 + b_{12}c_1c_2^2 + b_{20}c_1^2 + b_{21}c_1^2c_2 + b_{22}c_1^2c_2^2 \quad (4)$$

where $b_{h,k}$ are the function coefficients to be calculated.

The coefficients $a_{h,k}$ and $b_{h,k}$ are chosen to minimize the sum, S , of the squares of the differences between observed and predicted values:

$$S_1 = \sum_{i=1}^n \left(A_{1i} - \sum_{h,k=0}^2 a_{h,k} c_{1i}^h c_{2i}^k \right)^2 \quad (5)$$

$$S_2 = \sum_{i=1}^n \left(A_{2i} - \sum_{h,k=0}^2 b_{h,k} c_{1i}^h c_{2i}^k \right)^2 \quad (6)$$

In the present case c_{1i} represents the i -th concentration of NH_4^+ or ClO_3^- ; c_{2i} represents the i -th concentration of Na^+ or Br^- ; A_{1i} = i -th peak area of the NH_4^+ or ClO_3^- ; A_{2i} = i -th peak area of the Na^+ or Br^- .

The minimization is performed by solving the following equations:

$$\frac{\partial S_1}{\partial a_{h,k}} = 0 \quad \frac{\partial S_2}{\partial b_{h,k}} = 0 \quad (7)$$

where $h = 0, 1, 2$; $k = 0, 1, 2$.

Once the coefficient is computed, the so called inverse problem (that is the achievement of an approximation of the unknown concentrations \bar{c}_1, \bar{c}_2 from the knowledge of the areas, say \bar{A}_1, \bar{A}_2 of the two peaks) must be solved. This problem reduces to the solution of a 2×2 system of non linear equations:

$$\sum_{h,k=0}^2 a_{h,k} c_1^h c_2^k = \bar{A}_1 \quad (8)$$

$$\sum_{h,k=0}^2 b_{h,k} c_1^h c_2^k = \bar{A}_2 \quad (9)$$

which can be iteratively solved by means of the Newton–Raphson method.

Sixteen standard solutions covering the range 5–25 mg/l of both Br^- and ClO_3^- ions and 1–150 mg/l; 1–15 mg/l for Na^+ and NH_4^+ , respectively were used for optimization of the fitting functions.

Fig. 5 shows, in a three-dimensional plot, the quadratic surfaces obtained from the fitting of experimental data for $\text{ClO}_3^-/\text{Br}^-$ (Fig. 5a) and $\text{Na}^+/\text{NH}_4^+$ (Fig. 5b). To verify the validity of the calibration function, further samples at different concentrations have been analyzed (intermediate points).

As can be seen from these figures, in both the cases, all the experimental data (calibration “○” and intermediate “□” points) are exactly located on the surface plots. The enhanced curvature of the surface shown in Fig. 5b is related to the above discussed high influence of the sodium ion concentration with respect to the ammonium ion area evaluation, already evidenced by Fig. 2b.

In Table 1 the correlation coefficients r and the S values related to the fitting functions described by Eqs. (3) and (4) for the two cases under study are reported. In the same table the comparisons of the data obtained by the perpendicular at the valley or by the linear baseline methods are reported. It is worth noting that both methods lead to very similar results.

The low S value as well as the correlation coefficients r , practically unitary, are a good evidence of the validity of the proposed approach. As can be seen by the data shown in Fig. 1b, the higher S values observed for Na^+ are due to its higher peak area. The reliability of the method is confirmed by the good symmetry of the residuals with respect to the zero, as evidenced, by the example reported, in Fig. 6.

In Fig. 7a and b we report the observed concentrations vs. predicted values related to standard solutions prepared for both sodium/ammonium and chlorate/bromide ion couples. As can be seen, the predicted values perfectly fit the straight line of the observed ones. Moreover, Fig. 7c and d show that

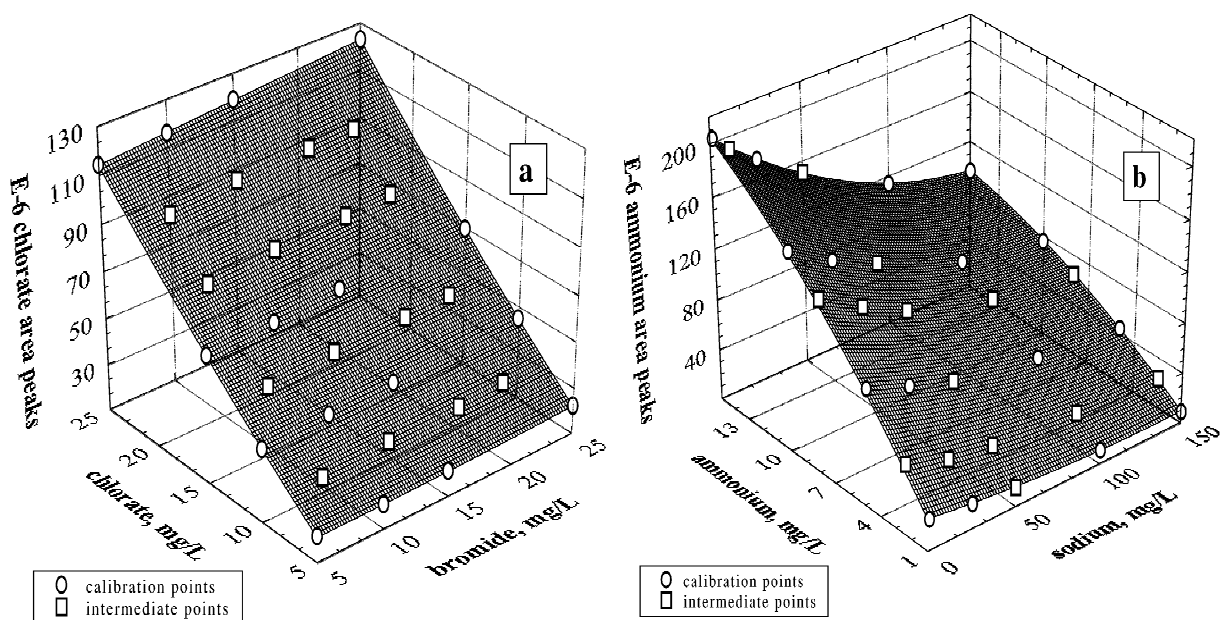


Fig. 5. Relation between the peak area of an ion vs. its and interferent ion concentrations: chlorate (a), ammonium (b).

most of the residuals are within the 95% of confidence, thus confirming the good agreement between experimental and theoretical data.

Table 2 summarizes the results obtained by analyses of solutions containing variable concentrations of the two ion couples.

4. Conclusions

Owing to the large variety of practical problems

Table 1

Comparison of the correlation coefficients and S values related of the fitting functions (3) and (4) for the sodium/ammonium and chlorate/bromide ion couples calculated by perpendicular at the valley and linear baseline methods (data relative to calibration standard solutions)

	Perpendicular		Linear "baseline"	
	r	S	r	S
ClO_3^-	0.99997	1.42	0.99991	1.48
Br^-	0.99998	0.84	0.99995	1.28
Na^+	0.99999	980 ^a	0.99999	1169 ^a
NH_4^+	0.99996	7.60	0.99992	9.75

^a The higher S values observed for sodium ion are related to its higher chromatographic response.

encountered in the chromatographic analysis, the selection of a suitable calibration method may constitute a difficult problem. The aim of this work was to develop a simple approach, based on empirical observation, for the determination of the concentrations of analytes leading to partially overlapped chromatographic peaks. This method can be successfully applied also in the case of analytes exhibiting strongly different chromatographic responses. As an example of this aspect, the sodium/ammonium ion couple was considered, where with a unique equation it was possible to quantify NH_4^+ in a really wide concentration range. However, it must be emphasized that in principle the empirical quadratic relationship could not be observed when the overlapping pair of compounds have different interactions with the stationary phase. In this case changes in both retention time and peak width could be present, negatively reflecting on the proposed model. Work is in progress to verify this aspect.

The main advantages of this approach are its artlessness toward other complicated methods for resolving partially overlapped peaks, especially in routine analyses, and the possibility to obtain immediately reliable results. The analytical data so obtained are practically independent of elution time fluctuations. The proposed method can be applied to

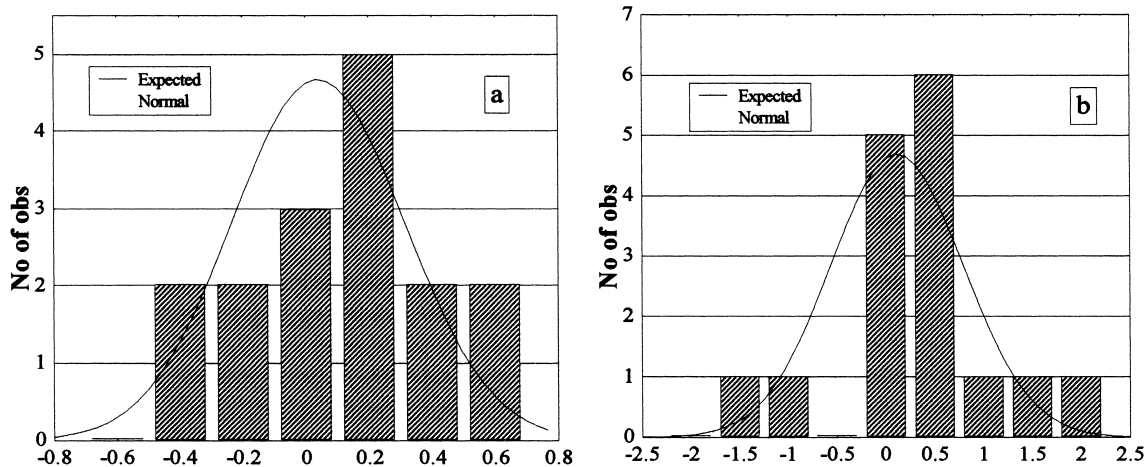


Fig. 6. Frequency distribution of the residuals for Eq. (3): chlorate (a) and ammonium (b) (data relative to calibration standard solutions).

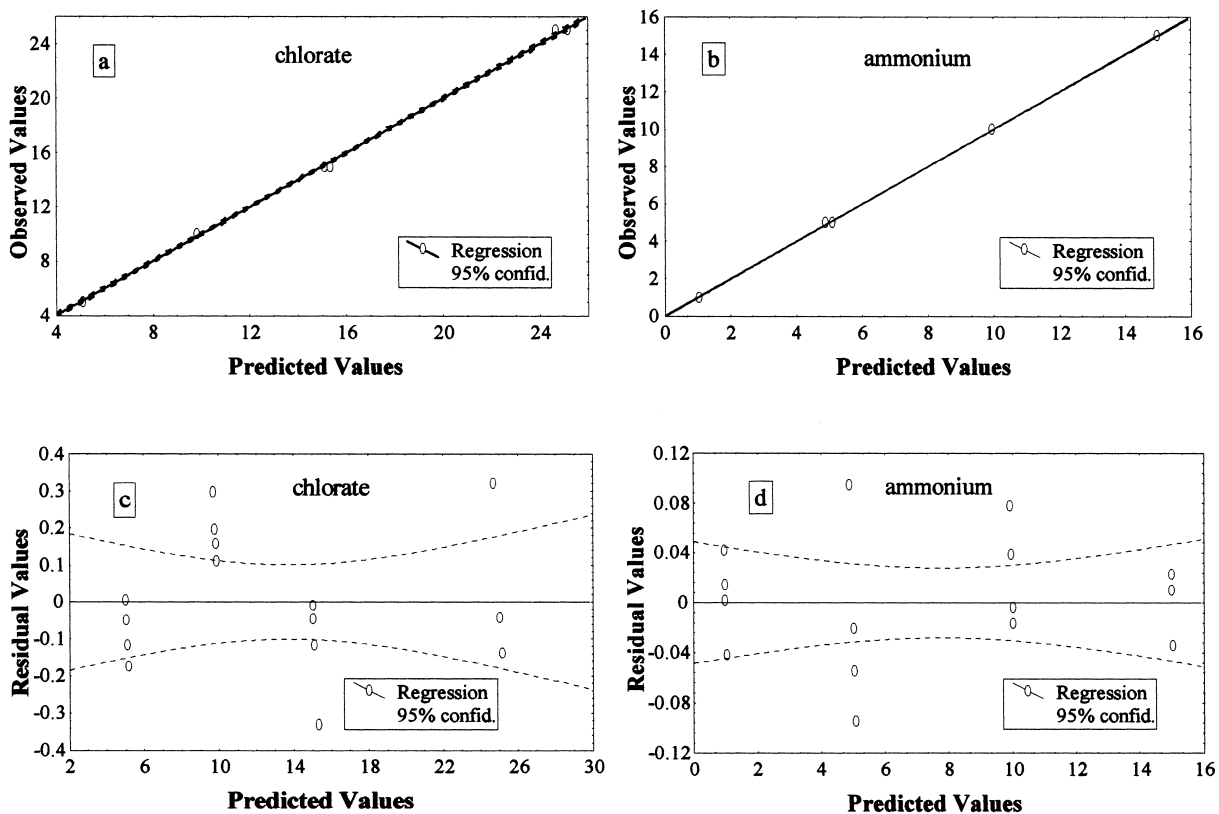


Fig. 7. Observed vs. predicted values (a, b) and residual vs. predicted values (c, d) for: chlorate and ammonium (data relative to calibration standard solutions).

Table 2

Observed and predicted concentration values for the couples: chlorate/bromide, sodium/ammonium (mean values obtained by three different measurements)

Br ⁻ observed (mg/l)	ClO ₃ ⁻ observed (mg/l)	Br ⁻ predicted (mg/l)	ClO ₃ ⁻ predicted (mg/l)	Na ⁺ observed (mg/l)	NH ₄ ⁺ observed (mg/l)	Na ⁺ predicted (mg/l)	NH ₄ ⁺ predicted (mg/l)
5.0	5.0	5.06	5.08	1.0	1.0	1.03	1.04
10.0	5.0	9.89	5.00	1.0	5.0	0.94	5.09
15.0	5.0	15.10	5.06	1.0	10.0	1.01	9.96
25.0	5.0	24.99	5.00	1.0	15.0	0.96	14.99
5.0	10.0	4.99	9.84	5.0	1.0	4.98	0.96
10.0	10.0	9.94	9.93	5.0	5.0	5.00	4.91
15.0	10.0	14.97	9.88	5.0	10.0	5.07	10.00
25.0	10.0	24.99	10.00	5.0	15.0	5.03	15.03
5.0	15.0	5.05	15.10	50.0	1.0	50.06	1.00
10.0	15.0	9.93	15.05	50.0	5.0	49.77	5.02
15.0	15.0	15.10	15.09	50.0	10.0	50.35	10.02
25.0	15.0	25.00	15.00	50.0	15.0	49.81	14.98
5.0	25.0	5.05	25.01	150.0	1.0	149.95	0.99
10.0	25.0	9.88	24.94	150.0	5.0	150.18	5.05
15.0	25.0	15.07	25.02	150.0	10.0	149.75	9.92
25.0	25.0	24.99	25.00	150.0	15.0	150.11	15.04

different chromatographic techniques (GC, HPLC, etc.). Its limits lie in the fact that the analytical data to be obtained must be inside those employed for calibration and that a just sketched valley must be present between the chromatographic peaks.

The method extension to multicomponent analysis as well as to cases in which any valley between the peaks is not detectable is currently in progress.

References

- [1] J. Weiss, Handbook of Ion Chromatography, Dionex, Sunnyvale, CA, 1986.
- [2] A.L.L. Duchateau, B.H.M. Munsters, G.T.C. Kwakkenbos, R.G.J. Van Leuken, J. Chromatogr. 552 (1991) 605.
- [3] P. Pastore, A. Boaretto, I. Lavagnini, A. Diop, J. Chromatogr. 591 (1992) 219.
- [4] C. Saigone, S. Kirchner, M. Legrand, Anal. Chim. Acta 203 (1987) 11.
- [5] U. Baltensperger, J. Hertz, J. Chromatogr. 324 (1985) 153.
- [6] M.J. Willison, A.G. Clarke, Anal. Chem. 56 (1984) 1037.
- [7] M. Miyazaki, K. Hayakawa, S.-G. Choi, J. Chromatogr. 323 (1985) 443.
- [8] K. Tanaka, T. Ishizuka, H. Sunahara, J. Chromatogr. 177 (1979) 21.
- [9] A. Need, C. Karmen, S. Sivakoff, A. Karmen, J. Chromatogr. 158 (1978) 153.
- [10] G. Schulze, C.Y. Liu, M. Brodowski, O. Elsholz, Anal. Chim. Acta 214 (1988) 121.
- [11] J.R. Torres-Lapasíó, J.J. Baeza-Baeza, M.C. García-Alvarez-Coque, Anal. Chem. 69 (1997) 3822.
- [12] I. Rodríguez, M.H. Bollaín, R. Cela, J. Chromatogr. A 750 (1996) 341.
- [13] L. Xu, I. Schechter, Anal. Chem. 69 (1997) 3722.
- [14] R.D. Bautista, A.I. Jiménez, F. Jiménez, J.J. Arias, J. Pharm. Biomed. Anal. 15 (1996) 183.
- [15] M.M. Galera, J.L.M. Vidal, A.G. Frenich, M.D.G. García, J. Chromatogr. A 778 (1997) 139.
- [16] J.M. Davis, Anal. Chem. 69 (1997) 3796.
- [17] A. Felinger, T. Pap, J. Inczédy, Talanta 41 (1994) 1119.
- [18] J.M. Davis, Anal. Chem. 65 (1993) 2014.
- [19] J.P. Foley, J.G. Dorsey, Anal. Chem. 55 (1983) 730.
- [20] M.S. Jeansonne, J.P. Foley, J. Chromatogr. Sci. 29 (1991) 258.
- [21] F. Hock, Chromatographia 2 (1969) 334.
- [22] H.A. Hancock Jr., L.A. Dahm, J.F. Muldoon, J. Chromatogr. Sci. 8 (1970) 57.
- [23] H.M. Gladney, B.F. Dowden, J.D. Swalen, Anal. Chem. 41 (1969) 883.
- [24] A.H. Anderson, T.C. Gibb, A.B. Littlewood, J. Chromatogr. Sci. 8 (1970) 640.
- [25] Y. Mori, J. Chromatogr. 66 (1972) 9.
- [26] J.T. Lundeen, R.S. Juvet Jr., Anal. Chem. 53 (1981) 1369.