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Resolution and quantitative determination of coeluted pesticide mixtures in liquid chromatography–thermospray mass spectrometry by multivariate curve resolution

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Abstract

Resolution and quantitative determination of a mixture of coeluted pesticides (carbofuran, propoxur and pirimicarb) in liquid chromatography–thermospray mass spectrometry by multivariate curve resolution is shown. Carbofuran and pirimicarb both exhibit an ion with m/z 239 in their thermospray mass spectrum. For the first compound this ion is attributed to the ammonium adduct $[M+NH_4]^+$ ion, whereas the corresponding ion of pirimicarb is the protonated molecule, $[M+H]^+$. A third carbamate, propoxur, was added because this compound partly coelutes with carbofuran under normal experimental conditions during multiresidue analysis of carbamates with C_{18} packed analytical columns. With this additional compound, the so-called suppression effects on the ion formation of these compounds in thermospray mass spectrometry and their quantitative resolution using chemometric methods were studied. © 1998 Elsevier Science B.V.

Keywords: Chemometrics; Multivariate curve resolution; Pesticides; Carbofuran; Propoxur; Pirimicarb; Carbamates

1. Introduction

In recent years there has been an extensive development of multivariate data analysis techniques in different branches of analytical chemistry and in particular in chromatography. Within these methods an important group of chemometric techniques have been proposed for the determination of peak purity, detection of interferences, resolution and quantitative estimation improvement [1–3]. Most of these works have been applied to liquid chromatography–diode array detection (LC–DAD) [4–7]. Less often these multivariate techniques have been applied to gas chromatography–mass spectrometry (GC–MS) [8–10] and even less to LC–MS [11,12]. There are several aspects that deserve special attention for LC–MS. The first important aspect to consider is data bilinearity, i.e., to asses whether a single pure analyte gives a rank one second order (two-way) data response. A second important aspect is to examine the noise structure, which owing to intrinsic characteristics of LC–MS is expected to have an important effect. Peak purity techniques depend on noise structure and on resolution of chromatographic coeluted peaks, specially in the case of strongly MS overlapped signals (common m/z ions). Finally, and specially important in analytical chemistry, is the aspect of quantitation of coeluted mixtures. In spite of the successful use of LC–MS as an identification and semiquantitative tool for complex mixture analysis, quantitation of coeluted mixtures deserves special attention in the case of interelement interferences in thermospray (TSP) interfaces.

In the present work, a recently proposed multi-

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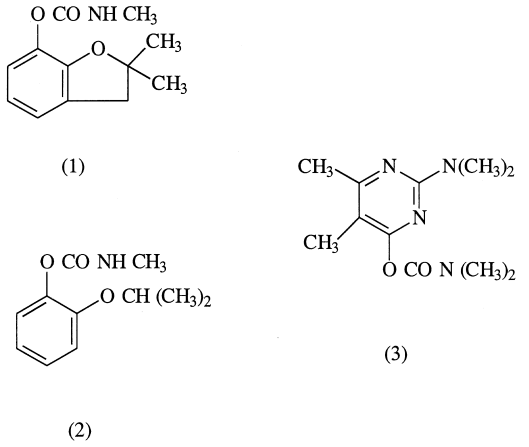

Fig. 1. Chemical structure of the studied compounds: (1) carbofuran, M_r 221.3; (2) propoxur, M_r 209.2; and (3) pirimicarb, M_r 238.3.

variate technique are investigated to solve the problems previously mentioned. Multivariate curve resolution (MCR) [13–15] has been applied for the analysis of peak purities, resolution and quantitation of LC coeluting compounds originating from real complex samples, utilizing LC–DAD [13–18]. Peak purity analysis of coeluting compounds in LC–TSP-MS from real experimental samples has been reported [12].

In the present work, three carbamate pesticides, carbofuran, propoxur and pirimicarb were used. The chemical structures of these compounds are depicted in Fig. 1. These compounds were selected for two reasons. First of all carbofuran and pirimicarb both exhibit an ion with m/z 239 in their TSP-MS spectrum. For the first compound this ion is attributed to the $[M+NH_4]^+$ ion, whereas the corresponding ion of pirimicarb is the protonated molecule, $[M+H]^+$ (Table 1). Secondly, a third carbamate, propoxur, was added because this compound partly coelutes with carbofuran under normal experimental conditions during multiresidue analysis of carbamates with C_{18} packed analytical columns. With this additional compound, the so-called suppression effects on the ion formation of these compounds in LC–TSP-MS can be studied.

Table 1
Main ions detected and most probable configuration for the three studied compounds

Compound	Ion	Configuration
Pirimicarb	239	$(M+H)^+$
Carbofuran	222	$(M+H)^+$
	239	$(M+NH_4)^+$
Propoxur	210	$(M+H)^+$
	227	$(M+NH_4)^+$

2. Experimental

2.1. Instrumentation

The TSP experiments were performed on an HP 5988A quadrupole mass spectrometer (Hewlett–Packard, Palo Alto, CA, USA), coupled to a HP Model 59970C data system for acquisition and data analysis. Full-scan mass spectra were acquired with a scan range of m/z 200–275 in the positive ion (PI) mode, for all ionization modes. The ion source and stem/tip temperatures were set at 250 and 135/208°C, respectively. The stem/tip temperatures corresponded to the point of 95% evaporation within the vaporizer, considered to be the optimum under the experimental set-up used [19].

An 1.0 ml/min carrier stream flow-rate of a 45% aqueous acetonitrile solution, containing 100 m*M* ammonium acetate, was delivered with a HP 1090 Series 1 liquid chromatograph (Hewlett–Packard, Waldbronn, Germany). Ten μl of sample was injected onto a 150×4.6 mm C_8 analytical column (Baker, Deventer, Netherlands) for the calibration studies. The injection volume was 20 μl for the studies using the mixture of the three compounds.

2.2. Experimental design

The full-scan range (m/z 200–275) was acquired with an scan speed of 214.5 atomic mass units per second. Every data point was obtained by averaging eight measured points.

In order to obtain rather good full scan TSP mass spectra, the concentration levels of the standard solutions, dissolved in 50% aqueous acetonitrile, between 5–100 mg/l were used. First, calibration curves of the separated compounds were performed injecting standard solutions at different concentration levels of each compound. For the studies with the samples containing mixtures of the three carbamate

Table 2
Composition of analyzed samples

Matrix[a]	PIR[b]	CAR[c]	PRO[d]
ms26	250	0	0
ms27	250	0	0
ms28	100	0	0
ms29	100	0	0
ms30	50	0	0
ms31	50	0	0
ms32	10	0	0
ms33	10	0	0
ms34	0	10	0
ms35	0	10	0
ms36	0	50	0
ms37	0	50	0
ms38	0	100	0
ms39	0	100	0
ms42	0	0	10
ms43	0	0	10
ms45	0	0	50
ms46	0	0	50
ms47	0	0	100
ms49	50.5	47.75	44
ms50	50.5	47.75	44
ms51	101	95.5	88
ms52	101	95.5	88
ms53	252.5	238.7	222
ms54	252.2	238.7	222
ms55	505	477.5	444
ms56	505	477.5	444
ms57	1010	955	880
ms58	1010	955	880
ms59	840	906	812
ms60	840	452	812
ms61	840	272	812
ms62	840	906	406
ms63	840	452	406
rns64	840	272	406
ms65	840	906	244
ms66	840	452	244
ms67	840	272	244
ms68	420	906	812
ms69	420	452	812
ms70	420	272	812
ms71	420	906	406
ms72	420	452	406
ms73	420	272	406
ms74	420	906	244
ms75	420	452	244
ms76	420	272	244
ms77	252	906	812
ms78	252	452	812
ms79	252	272	812
ms80	252	906	406
ms81	252	452	406
ms82	252	272	406
ms83	252	906	244
ms84	252	452	244
ms85	252	272	244

[a] Matrix identification; [b] pirimicarb; [c] carbofuran; [d] propoxur.

at various concentration levels, an experimental design was applied, and 27 different samples were injected (Table 2). All samples were analysed in duplicate. For example, when the concentration of pirimicarb was maintained constant (42, 21 or 12.6 mg/l) the concentrations of propoxur (40.6, 20.3 and 12.2 mg/l) and carbofuran (45.3, 22.6 and 13.6 mg/l) were varied. Thus, with a pirimicarb concentration of 42 mg/l, nine samples with different concentrations of propoxur and carbofuran were prepared and injected. At three concentration levels of pirimicarb this results in 27 samples.

Raw experimental data were transfered from HP PASCAL internal format to ASCII PC and to MATLAB [20] internal format using a laboratory-written QUICK BASIC DOS program.

3. Method

3.1. Rank analysis, noise structure and peak purity

Rank analysis is used for the investigation of the data structure and to test the initial assumption of data bilinearity, i.e., that the LC–MS response of a pure single analyte is a rank one data matrix. The basic tool for the investigation of the ''chemical'' rank is the determination of the number of largest singular values related with chemical sources of data variation (and not with noise) obtained in the singular value decomposition and/or principal component analysis (PCA) [1–3] of the individual data matrices. From the visual inspection of the plots of the first ten singular values and from the amount of explained data variance, and the assumption that most of the data variance is of chemical nature, the number of chemical contributions is initially estimated. For the analysis of single analyte samples this number must be one if the condition of data bilinearity holds. Also, in that case, the amount of data variance explained by one single component should be high. If data is bilinear, the PCA lack of fit, or the amount of variance not explained by the deduced chemical components, would give an estimation of the noise level of the experimental data. When the data are nonbilinear or there is a large noise contribution, the lack of fit becomes high. These two cases, nonbilinearity or large noise contributions are difficult to

distinguish, specially if nonbilinearity implies a nonlinear behavior of the LC–MS interfacing process which cannot be explained by a reduced number of linear contributions.

Noise structures and peak purity can also be studied by evolving factor analysis (EFA) [21–24] and other related methods [4,5]. From the plots of the singular values or eigenvalues evolving with the chromatographic elution, the systematic noise contributions can be detected. Heteroscedasticity noise has been shown to be present in DAD [3,4]. EFA with a fixed size moving window with some data offset pretreatment has been shown to be useful in detecting this type of problem [3,5]. However, detection of heteroscedasticity is also related to resolution of strongly coeluted minor components or impurities and special caution has to be taken in the interpretation of such a plot [1]. As peak purity multivariate detection methods assume data bilinearity and homoscedastic noise, departures from these conditions cause problems.

3.2. Resolution of LC coeluted mixtures

Mixtures of chemical compounds which cannot be easily separated by chromatographic means give coeluted peaks. Using a highly selective detection method, like MS, usually allows the identification and quantitation of the coeluted compounds. However, as was mentioned previously, some problems can occur when these coeluting compounds exhibit similar MS signals or because of matrix effects (related with the TSP). MCR methods [13–15] have been shown especially suitable to resolve the pure components present in unknown coeluted mixtures. The application of the proposed method to resolve real LC–DAD data problems has been described in detail in previous works [16–19]. The basic assumption of the resolution procedure is the fulfilment of the linear model, described by the equation:

$$\mathbf{D}_i = \mathbf{C}_i\mathbf{S}^{\mathbf{T}} + \mathbf{E}_i$$

where $\mathbf{D}_i$ is the data matrix obtained in one chromatographic run, $\mathbf{C}_i$ is the matrix of the concentration profiles of the coeluted compounds, $\mathbf{S}^{\mathbf{T}}$ is the matrix of MS pure spectra of these coeluted compounds and $\mathbf{E}_i$ is the residual error matrix containing the data variance not explained by the compounds described in $\mathbf{C}_i$ and $\mathbf{S}^{\mathbf{T}}$. The goals of the proposed method when it is applied to a single chromatographic run are: (1) estimation of the number of coeluted compounds; and (2) resolution of these coeluted compounds, i.e., obtention of matrices $\mathbf{C}_i$ and $\mathbf{S}^{\mathbf{T}}$. Two key steps of this procedure when applied to strongly coeluted LC–MS experimental data are: (1) the initial estimation of the LC elution profiles or of the MS spectra of the unknown components in their mixtures using EFA derived methods [21–24] or other pure variables detection methods like SIMPLISMA [25]; and (2) the optimization of these initial profiles by using an alternating least squares (ALS) optimization of the elution and spectra profiles [26]. The optimization search is performed using some natural constraints known to be present in the experimental data like nonnegativity and chromatographic peaks unimodality. As MS is a highly selective detection technique (except in the case of coeluting compounds with similar ions), resolution is usually achieved much faster than with other spectroscopic detection methods like UV–Vis or Fourier transform IR.

Simultaneous analysis of several correlated data matrices obtained in different chromatographic runs of the same system by using the proposed MCR has been shown to be a very powerful method to improve the resolution of strongly coeluted (poorly resolved) chromatographic peaks [13–15,26]. In that case a set of

$$\mathbf{D} = \begin{bmatrix} \mathbf{D}_1 \\ \mathbf{D}_2 \\ \cdots \\ \cdots \\ \mathbf{D}_n \end{bmatrix} = \begin{bmatrix} \mathbf{C}_1 \\ \mathbf{C}_2 \\ \cdots \\ \cdots \\ \mathbf{C}_n \end{bmatrix} \mathbf{S}^{\mathbf{T}} + \begin{bmatrix} \mathbf{E}_1 \\ \mathbf{E}_2 \\ \cdots \\ \cdots \\ \mathbf{E}_n \end{bmatrix} \qquad (2)$$

or in abbreviated form

$$\mathbf{D} = \mathbf{C}\mathbf{S}^{\mathbf{T}} + \mathbf{E} \qquad (3)$$

where $\mathbf{D}$ is the augmented data matrix, keeping the data matrices $\mathbf{D}_i$ one on top of each other keeping their columns (m/z ions) the same for all of them, and $\mathbf{D}$ has a number of rows equals to the total number of acquired spectra in the different chromatographic runs (elution times), $\mathbf{C}$ is the augmented concentration matrix, $\mathbf{S}^{\mathbf{T}}$ the spectra matrix, and $\mathbf{E}$ the augmented residual error.

A more detailed description and validation of the proposed curve resolution method when it is applied to the simultaneous analysis of several chromatographic runs has been given elsewhere [13–18,26].

3.3. Calibration curves and quantitation of coeluted mixtures

Calibration and quantitation of coeluted mixtures is possible when multiple chromatographic runs of standard and unknown samples are simultaneously analysed using the proposed MCR method [16]. In order to check the conditions under which the calibration and quantitation of the three studied analytes, pirimicarb, carbofuran and propoxur, in their mixtures are better, the following cases are studied:

1. Calibration of individual analytes in the absence of coeluted compounds: a set of chromatographic runs of samples containing a single analyte at different concentrations are simultaneously analysed. Each chromatographic run gives a data matrix $\mathbf{D}_i$; the set of data matrices obtained in the different chromatographic runs give a set of data matrices which are arranged in the new augmented column-wise data matrix **D** (Eq. (2)). From the areas of the resolved elution profiles in the analysis of standard samples with known concentration of the analyte, calibration curves are obtained for each one [16].
2. Calibration of individual analytes in the presence of coeluted compounds (interferents) at constant concentration: in this case, different samples containing one analyte at varying concentration and the other two components at constant concentration acting as interferents, are analysed. A new augmented data matrix **D** (Eq. (2)) is built up from the individual data matrices obtained in the chromatographic analysis of the different mixture samples. Nine different data treatments are performed at three different concentration levels of the interferents. Each data treatment implies the simultaneous analysis of three mixture data matrices containing the analyte at three different concentration levels and the other two compounds at constant concentration.
3. Analysis of samples with the individual analytes at constant concentration in the presence of coeluted compounds (interferents at varying concentration): in this case, different samples of one analyte at constant concentration and the other two components at varying concentration acting as interferents, are analysed. A new augmented data matrix **D** (Eq. (2)) is built up from the individual data matrices obtained in the chromatographic analysis of the different mixture samples. Three different data treatments are performed at three different concentration levels of the analyte. Each data treatment implies the simultaneous analysis of nine data mixture matrices containing the analyte at one constant concentration level and the other two compounds at three different concentration levels.

There are two possible approaches for the simultaneous analysis and calibration of the individual mixture data matrices (cases 2 and 3 above). In the first approach, the set of mixture matrices is simultaneously analysed with three matrices each one containing only one of the three different analytes. In this way, the information about each of the analytes is provided independently in each of the three pure analyte matrices. In the second approach, the set of mixture matrices is simultaneously analysed with only one pure analyte data matrix. This means that only the information of one of the analytes is provided in its pure data matrix, whereas the information about the other two components is not given independently and hidden (not resolved) in the mixture matrices. This second case is of course the more interesting one since it corresponds to the analysis of one analyte in an unknown mixture in the presence of strong interferences.

4. Results

4.1. Rank analysis, noise structure and peak purity

Table 3 shows the results of PCA of the data matrices obtained in the LC–MS analysis of pure analyte samples at different concentrations. The relative magnitudes of the first singular values and of the PCA lack of fit values obtained when a single component is considered show different situations depending on the compound studied. Pirimicarb behaves quite well at high concentrations (250 and

Table 3
Multivariate analysis of pure analyte samples

Data[a]	Concentration[b]	PC1[c]	PC2[3]	% PCA fit[d]	% ALS fit[d]
ms26	250	1.82	0.07	4.88	4.89
ms27	250	2.70	0.05	2.52	2.85
ms28	100	1.39	0.04	3.89	4.03
ms29	100	1.49	0.08	6.34	6.41
ms30	50	0.56	0.04	9.63	9.76
ms31	50	0.56	0.04	8.19	8.26
ms32	10	0.12	0.04	34.5	35.9
ms33	10	0.12	0.04	32.7	33.7
ms38	100	4.04	0.41	12.2	12.3
ms39	100	3.95	0.41	12.8	13.0
ms36	50	1.97	0.39	21.8	22.7
ms37	50	2.12	0.37	19.5	19.9
ms34	10	0.76	0.33	46.1	51.2
ms35	10	0.70	0.29	44.2	53.9
ms47	100	1.24	0.04	3.90	3.95
ms46	50	1.25	0.04	3.88	3.92
ms45	50	0.88	0.04	5.04	5.10
ms43	10	0.11	0.04	35.6	37.6
ms42	10	0.12	0.04	30.8	31.7

[a] Data matrix identification (see Table 2); [b] amount injected in ng; [c] first (PC1) and second (PC2) principal components of the data matrix; [d] lack of fit $lof = 100\sqrt{\frac{\sum (d_{ij}^{*} - d_{ij})^2}{\sum (d_{ij})^2}}$ where d_{ij} are the experimental data for sample i and m/z ratio channel j and d_{ij}^{*} are the corresponding reproduced data values calculated using principal component analysis (PCA *lof*) or using alternating least squares (ALS *lof*).

100 ng), giving experimental data matrices with a first large singular value. Therefore, the pirimicarb data matrix can be described reasonably well considering only one single component (around 95% of the data variance), which is in agreement with what is expected for a pure analyte linear response data matrix with a low noise level. However, when the pirimicarb data matrices at lower concentration are analysed by PCA, a considerable growth of the second singular value and an increase of the PCA lack of fit with one component (up to 34% for 10 ng of pirimicarb injected) is observed. This means that at these low concentrations the LC–TSP-MS chromatographic system does not behave linearly and that the noise level becomes important.

The results obtained for carbofuran and propoxur are also given in Table 3. Whereas propoxur gives similar results than pirimicarb, carbofuran shows a more complex behavior. The second singular value of the carbofuran matrices is always very large and the PCA lack of fit considering only one component is always large (more than 10%), even at high concentrations (100 ng) of the analyte (carbofuran). These differences in the behavior between pirimicarb and propoxur on one side and carbofuran on the other side are explained by strong influences of various chemical ionisation parameters such as: ion source temperature, ion source pressure and the presence of more basic compounds (Fig. 4, see below) [19,28].

Peak purity is investigated by means of EFA [4,5,21–23] techniques. In Fig. 2 the EFA plot of a pure analyte data matrix (100 ng of pirimicarb) is shown. Three regions are distinguished in this plot: a first region where the first component (largest singular value) emerges, both in the forward (curve 1f) and in the backward (curve 1b) analysis; a second intermediate region where three middle size components are evolving (2f, 3f and 4f) in forward analysis and three middle size components (2b, 3b and 4b) are evolving in the backward analysis; a third region, where the rest of evolving singular

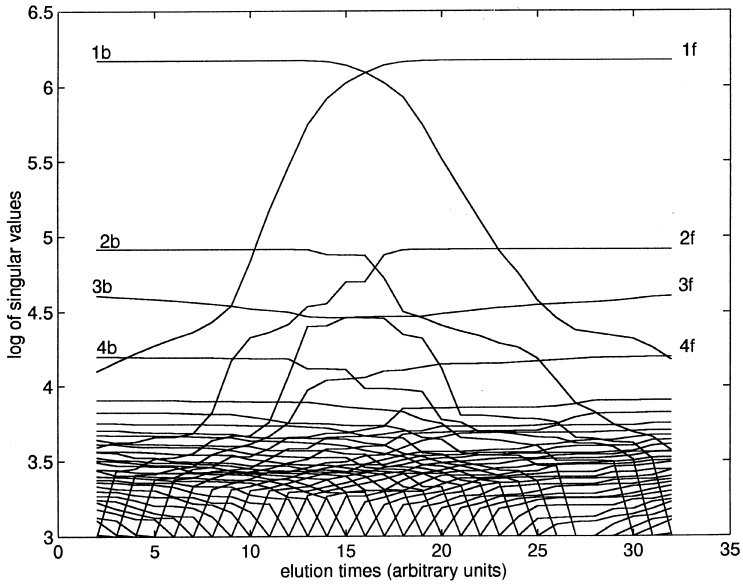

Fig. 2. Evolving factor analysis plot (see text and Refs. [22–24]) of 100 ng of pure pirimicarb data matrix.

values appear at the bottom of the plot. The first region is the region where most of the chemical information is assumed to be present, i.e., the pure TSP-MS pirimicarb signal during its chromatographic elution. The singular values of the third region are associated with the experimental noise, mostly uniformly distributed error. The more difficult region to interpret is the intermediate one. The appearance of at least three singular values in this region can be related with a nonlinear behavior of the system or, with a nonrandom (systematic) noise or with instrumental signal contributions. The two problems are difficult to distinguish. As shown in Fig. 2 for a pure pirimicarb matrix at concentration equal to 100 ng, these intermediate effects are already present. The same or even worse effects can be detected in the case of carbofuran and propoxur pure analyte samples. Since EFA analysis of the pure analyte samples already gives such complicated patterns, the peak purity analysis of samples with mixtures of the three components using EFA techniques or any other peak purity technique will be extremely difficult, especially when the impurities are at low concentrations or contribute little to the global signal. In these difficult cases, instead of trying peak purity by a mathematical method, it is better to perform the peak purity analysis simultaneously to the curve resolution of the system using a method which combines mathematical modeling with personal judgment based on natural constrains.

4.2. Resolution of LC coeluted mixtures

Since the information provided by MS is selective, initial estimations of the pure MS spectra of the three coeluted components are more easily obtained by detection of the purest variables methods [25] than by EFA methods [21–24]. The results finally obtained however, were independent of the initial estimations provided either by purest variable detection methods or by EFA based methods.

In Fig. 3, the resolved elution profiles of the three analytes, pirimicarb, propoxur and carbofuran are given for three mixtures of them simultaneously analysed. They were obtained using the proposed ALS MCR method [13–18,26] when it is applied simultaneously to the three different mixture data matrices. Although strong coelution is observed in the three cases, resolution of the three compounds is attained, even for pirimicarb and carbofuran, which share the ion at m/z 239. Whereas carbofuran starts elution first, pirimicarb and propoxur give totally embedded peaks [27]. In this case, however, their

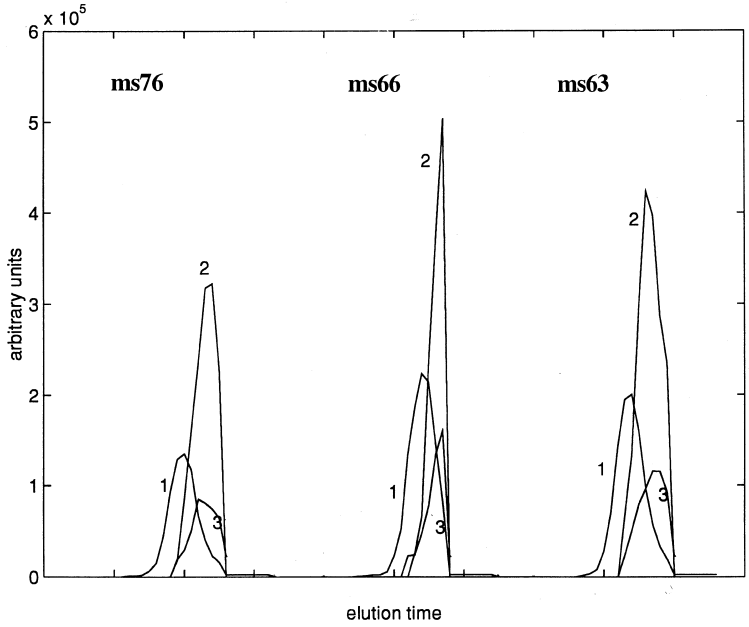

Fig. 3. Resolved elution profiles of the three coeluted compounds, (1 = carbofuran, 2 = pirimicarb, 3 = propoxur) at different concentrations in three different mixtures. The section of each analyzed chromatogram including the three coeluted peaks is given. *x*-Axis gives elution times in arbitrary units.

resolution is facilitated because their MS spectra do not overlap.

4.3. Calibration curves and quantitation of coeluted mixtures

1. Calibration of individual analytes in the absence of coeluted compounds: in the three studied cases (analysis of pirimicarb, carbofuran or propoxur), the calibration curves showed a good linear trend (Table 4). Although the sensitivity (slope of the calibration lines) for carbofuran at lower concentrations is higher than for pirimicarb and propoxur, the signal linearity of carbofuran degrades much faster than the signals for the other two analytes, pirimicarb and propoxur. Ordinate of the calibration lines provides a rough estimation of detection limits; in this case the values obtained are in agreement with previously estimated detection limits in LC–TSP-MS in PI mode [7].
2. Calibration of individual analytes in the presence of coeluted compounds at constant concentration:

Table 4
Correlation between concentration and peak areas

	Carbofuran			Pirimicarb			Propoxur		
	R^2	Max	Min	R^2	Max	Min	R^2	Max	Min
1	0.99	100	10	0.98	100	10	0.96	100	10
2	0.48	45.3	13.6	0.87	42	12.6	0.96	12.2	40.6
3	0.32	45.3	13.6	0.87	42	12.6	0.91	12.2	40.6

(1) Calibration of individual analytes in the absence of coeluted compounds; (2) calibration of individual analytes in the presence of coeluted compounds (interferents) at constant concentration; (3) calibration of individual analytes at constant concentration in the presence of coeluted compounds (interferents) at varying concentration.

R^2, Correlation between concentration and peak areas in the calibrations; Max, maximum concentration in the calibration samples sets; Min, minimum concentration in the calibration sample sets.

in Table 4 the results of the calibration of each analyte in the presence of the other two coeluted compounds as interferents at constant concentrations are given. Good correlation between the peak areas and the concentrations of the analyte at varying concentrations are observed for pirimicarb and propoxur. Worse quantification is observed, however, for carbofuran. Very similar results are obtained either for the simultaneous analysis including only the pure analyte matrix or for the simultaneous analysis including the three pure analyte matrices. This showed that in this case the resolution and the quantitation procedure were not affected by the inclusion or not of the matrices of the pure compounds acting as interferents.

3. Analysis of samples with the individual analytes at constant concentration in the presence of coeluted compounds interferents at varying concentration: in this case, also, a linear correlation between peak areas and concentrations of the analyte was observed for both pirimicarb and propoxur (Table 4). Carbofuran, again, gave a much worse correlation. As in the previous case, the resolution and quantitation procedure was not affected by the inclusion or not of the pure matrices of the compounds acting as interferents.

5. Discussion

The formation of ions in TSP-MS, using an ammonium acetate buffer with or without an external electron source (filament or discharge needle), is considered to be mainly due to gas-phase ion–molecule reactions between the protonated ammonium ion, $[NH_4]^+$, and a neutral molecule. This reaction is schematically depicted in Fig. 4. Most often, depending on the proton affinity of the compound, the ammoniated adduct ion and/or the protonated molecule are observed in their TSP mass spectra. The relative abundances of these typical ions can be strongly influenced by a variety of factors, e.g., the ion source geometry, temperature, and the partial pressure of both the ammonium ion and the target compounds [28,29]. This, especially, holds true for the carbamate pesticide carbofuran. In previous studies, it was observed that lowering of the ammonium acetate concentration from 100 to 1 m*M* resulted in a change of the base peak in the TSP mass spectrum. At high ammonium acetate concentration the ammoniated adduct ion with m/z 239 is the base peak, whereas the protonated molecule with m/z 222 takes over at lower concentration levels. Also large differences in the relative abundances of these ions were encountered between 14 different types of commercially available TSP interfacing systems. Therefore, careful control and optimization of these factors, in order to obtain reproducible data is needed.

These expectations were confirmed by the TSP data. That is, increase of carbofuran concentration, keeping the concentrations of pirimicarb and propoxur constant, and analysing the ammoniated ion with m/z 239, did not result in linear increase in the ion intensity (Table 4). These results are in contrast to the ideal situation with only carbofuran in the ion source (see Table 4), and can be explained by the strong sensitivity of carbofuran for pressure differences [28,29] together with the presence of more than one compound at the same time in the ion source. That is, the equilibrium of the protonation reaction under these conditions can differ significantly between the two experimental conditions,

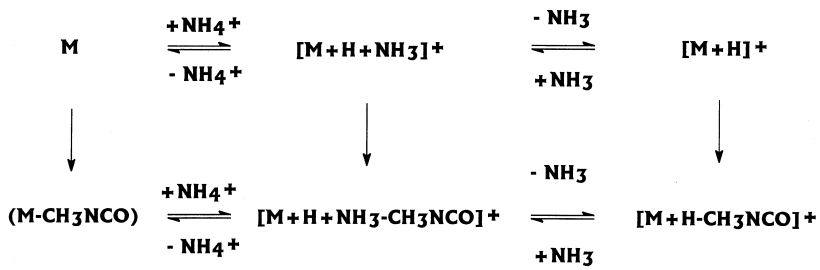

Fig. 4. Possible origin of ion signals in the ammonia positive chemical ionization mass spectra of carbamates.

generating variations in the relative abundances of the ions formed. Thus, protonation or ammonium adduct formation of the compound in the mixture with the lowest proton affinities is not complete. The compounds with the higher proton affinities and with an reaction equilibrium completely shifted to the right do not show such a behavior. Consequently, the calibration curves of propoxur and pirimicarb should be linear under all experimental conditions performed in this study. Indeed, the calibration curve for propoxur under ideal and coelution conditions was linear (Table 4). For this compound the ammoniated adduct ion with m/z 227 is monitored. The relative abundance of its protonated molecule, with m/z 210, is typically below 5% of the base peak. For pirimicarb, only exhibiting a protonated molecule with m/z 239 in its TSP-MS spectrum, linear calibration curves were obtained under all experimental conditions (Table 4).

These results show that the proposed MCR method is capable of distinguishing between the presence of two compounds with the same mass which are coeluting. More important is the observation that significant change of the relative abundances of target compounds are influenced by the presence of coeluting compounds. This especially is of importance when short analytical columns (loss of selectivity), typically between 1 and 4 cm, are used in combination with MS–MS (tandem MS) detection.

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References

[1] F. Cuesta Sánchez, B. van den Bogaert, S.C. Rutan, D.L. Massart, Chemometr. Intell. Lab. Syst. 34 (1996) 139–171.
[2] J.K. Strasters, H.A. Billet, L. de Galan, B.G.M. Vandeginste, J. Chromatogr. 499 (1990) 499–522.
[3] D.W. Osten, B.R. Kowalski, Anal. Chem. 56 (1984) 991–995.
[4] H.R. Keller, D.L. Massart, Anal. Chim. Acta 246 (1991) 379–390.
[5] H.R. Keller, D.L. Massart, J.O. De Beer, Anal. Chem. 65 (1993) 471–475.
[6] M.J.P. Gerritsen, H. Peters, B.G.M. Vandeginste, G. Kateman, Chemometr. Intell. Lab. Syst. 18 (1993) 205–219.
[7] I. Sakuma, N. Takai, T. Dohi, Y. Fukui, A. Ohkubo, J. Chromatogr. 506 (1990) 223–243.
[8] L. Roach, M. Guilhaus, Org. Mass Spectrom. 27 (1992) 1071–1076.
[9] W. Winding, S.A. Liebman, M.B. Wasserman, A.P. Snyder, Anal. Chem. 60 (1988) 1503–1510.
[10] A.R. Allan, J. Roboz, Rapid Commun. Mass Spectrom. 2 (1988) 246–249.
[11] D.K. Bryant, M.D. Kingswood, A. Belenguer, J. Chromatogr. A 721 (1996) 41–51.
[12] D. Lincoln, A.F. Fell, N.H. Anderson, D. England, J. Pharm. Biomed. Anal. 10 (1992) 837–844.
[13] R. Tauler, Chemometr. Intell. Lab. Syst. 30 (1995) 133–146.
[14] R. Tauler, D. Barceló, Trends Anal. Chem. 12 (1993) 319–327.
[15] R. Gargallo, R. Tauler, F. Cuesta Sànchez, D.L. Massart, Trends Anal. Chem. 15 (1996) 279–286.
[16] S. Lacorte, D. Barceló, R. Tauler, J. Chromatogr. A 697 (1995) 345–355.
[17] R. Tauler, S. Lacorte, D. Barceló, J. Chromatogr. A 730 (1996) 177–183.
[18] R. Tauler, G. Durand, D. Barceló, Chromatographia 33 (1992) 244–254.
[19] M. Honing, D. Barceló, B.L.M. van Baar, U.A.Th. Brinkman, Trends Anal. Chem. 14 (1995) 496.
[20] MATLAB, version 4.2c, The Math Works, Natick, MA, 1994.
[21] M. Maeder, A.D. Zuberbuehler, Anal. Chim. Acta 181 (1986) 287–291.
[22] M. Maeder, A. Zilian, Chemometr. Intell. Lab. Syst. 3 (1988) 205–213.
[23] M. Maeder, Anal. Chem. 59 (1987) 527–530.
[24] H.R. Keller, D.L. Massart, Chemometr. Intell. Lab. Syst. 12 (1992) 209–224.
[25] W. Winding, J. Guilment, Anal. Chem. 63 (1991) 1425–1432.
[26] R. Tauler, A. Smilde, B.R. Kowalski, J. Chemometr. 9 (1995) 31–58.
[27] Y. Liang, O. Kvalheim, Anal. Chim. Acta 276 (1993) 425–440.
[28] A.M. Honing, D. Barceló, J. Slobodnik, M.E. Jager, B.L.M. van Baar, U. Brinkman, J. Chromatogr. A 712 (1995) 21.
[29] D. Volmer, K. Levsen, M. Honing, D. Barceló, J. Abian, E. Gelpí, B.L.M. van Baar, U.A.Th. Brinkman, J. Am. Soc. Mass Spectrom. 6 (1995) 656.