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Develop of a multiway chemometric-based analytical method fulfilling regulatory identification criteria: Application to GC–MS pesticide residue analysis☆

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ABSTRACT

The proposed procedure is described by applying it to develop an analytical method which fulfils the SANCO specifications. Nevertheless, the procedure would be valid for any other legal specification that requires the identification of the analyte by means its *m*/*z* values and retention time. To demonstrate the procedure, three herbicides (simazine, Sz; atrazine, Az; propazine, Pz), with terbuthylazine, Tz, as internal standard (IS) have been analysed by gas chromatography with mass spectrometry detection (GC/MS). The procedure consists of the following steps: (i) To record the data in the full scan mode (201 *m*/*z* ratios). (ii) To select four characteristic ions which make possible the unequivocal identification of each triazine according to the criteria established in the Document SANCO/12495/2011 by means of principal components and hierarchical clustering of variables; (iii) To build a calibration based on the PARAFAC decomposition with the data recorded in SIM mode at the four *m*/*z* ratios selected for each triazine. Afterwards several figures of merit have been evaluated. Bearing in mind that triazines are one of the most frequently used group of herbicides in agriculture and atrazine and simazine are included in the list of priority substances in Annex II of Directive 2008/105/EC, in this work, these analytes have been analysed in three natural waters. Prior to determination by gas chromatography with mass spectrometry detection (GC/MS) a step with solid phase extraction (SPE) has been carried out. The calibration set is made up of 40 standards 33 are external standards prepared in acetone and seven matrix matched prepared in deionised water subjected to the SPE procedure. Moreover, each kind of water, stream, well, and river, is analysed both unspiked and spiked. For the triazine determination, the second order PARAFAC advantage allows the use of samples prepared in acetone together with those prepared in deionised water subjected to SPE. The decision limit, CCα, and the capability of detection, CCβ, are calculated according to ISO 11843-2, assessing the false positive and false negative. The *m*/*z* ratios chosen fulfils the SANCO identification criteria and also the spectrum obtained in the PARAFAC decomposition, which is common in all samples for each triazine. However, when the same experimental data are used to carry out a univariate calibration with the abundance of the base peak of each triazines, a lot of samples lie outside the permitted tolerances depending on the reference experimental spectra used, despite the fact that all of them have a triazine content above the detection limit. Also, the PARAFAC calibration allows us to detect the test samples which are not similar to the calibration samples and in this way their mistaken quantification is avoided.

1. Introduction

The SANCO document is the watchdog for plaguicide residues in the Eurpoean Union. The document describes the requirements necessary for quality control to establish the validity of the data used for checking maximum permitted residue levels, execution, actions and/or evaluation of consumer exposure to plaguicides. The first edition of the document SANCO (designed as 7826/VI/97) was published in 1997 and since January 2012 has been in force in its latest edition [1]. At the same time, was presented a guide elaborated by the AOAC/FAO/IAEA/IUPAC expert consultation about the analysis of organic chemicals at trace-level concentrations [2]. The annex of SANCO contains representative matrices, both of vegetable and animal origin, in which pesticide residues must be controlled.

Triazines are plaguicides belonging to the herbicide group and are used to control scrub. The great diversity and increase in

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agricultural activity as led to the wide use of herbicides. The chloro-triazines were widely introduced in the fifties. Simazine, Sz, propazine, Pz, and atrazine, Az, are currently among the most used triazines. It is generally necessary to control the use of triazine pesticides since their residues can contaminate both underground and surface water, making their way into water for human consumption. Indeed the bibliography points to papers [3] which consider these substances to be cancerigenous, especially simazine and atrazine, as well as including them in lists of possible endocrine disruptors [4,5]. The polarity of these herbicides means they can be dissolved in water, which facilitates their dispersal and environmental contamination. It is for this reason that they have been classified as pesticides for restricted use and permitted limits have been established by the USA (EPA) and by the European authorities. Both atrazine and simazine are to be found in the priority substances list in water policies outlined in Annex II of Directive 2008/105/EC [6], which also establishes environmental quality regulations for these priority substances (Annex I). The regulations laid down in this Directive were to have been set out in national laws of the European countries before July 2010, date by which the MRLs of these substances were to be fixed according to the matrix in which they are analysed, at 0.05, or, 0.1 mg kg^{-1} depending on the analytical matrix used.

The bibliography indicates studies carried out with HPLC with ultraviolet spectrophotometry, for example in Ref. [7], where the determination of pesticides is performed in tomatoes. In Ref. [8], diphasic dialysis as an extraction method for the analysis of triazines in milk (infant formulas) has been set up. But the pesticide residues have generally been analysed by gas chromatography with mass spectrometry as detection technique, in different matrices as: soil [9–12], juice [13] and honey [12,14]. The most widely used pre-treatment for samples in the analysis of these compounds has been solid phase extraction (SPE). In Ref. [15] a revision of this technique was made as well as different methods for the analysis of pesticide residues in water. Recently in 2010 in Ref. [16] use a chromatographic method with detection by mass spectrometry in tandem MS/MS, with solid phase extraction, SPE, for the quantification and confirmation of Sz, Az and Tz together with the eight most important products of their degradation in surface and waste water. Actually, solid-phase microextraction (SPME) [17] is the most widely employed technique for the extraction of these because of its suitability in aqueous media and the low detection limits achieved [18–22]. SPME is also used to determine these substances in matrices other than water [23–25]. The differences in the detection limits achieved according to the extraction technique chosen can be found in a review from 2000 which contains 43 references to separation methods for pesticides with amine groups in biological samples [26]. Table S1 of supplementary material gives a summary of the analytical matrices, ions selected and extraction and analytical methods used in the papers referred to earlier. It also shows the recovery achieved in each case and the detection limit, LOD, obtained, in all cases, without evaluating probabilities of false negative or false positive. In most of these articles, there is no mention made of how the m/z ions used in the determinations are selected, identifying the compounds solely by means of analyte retention times.

Currently European legislation regulates the authorisation, control and to a lesser extent the use of these types of product, establishing a complete process for the authorisation and evaluation of their active components. Az was the most widely used and most representative of all the s-triazines. Commercial use of this herbicide was prohibited in the European Union on environmental grounds [27], hence its control is of great importance. In 1998 EU legislation established 0.1 $\mu g\,L^{-1}$ as the maximum permitted concentration for individual triazines and 0.5 $\mu g\,L^{-1}$ for the sum of them all in drinking water [28]. European legislation in the field of water policy was governed by the Water Framework Directive 2000/60/EC [29] between 2000 and 2008 when it was amended. In 2001 the European Parliament Decision 2455/2001 [30] included the first list of "Priority Substances" for water policies: Az and Sz were marked in this list as substances to be studied as "priority dangerous substances". Later in 2008 were amended [6] and these triazines disappeared in the list as dangerous substances but they remained in the priority list with maximum permitted concentrations in surface waters. These directives formed the basis for the remaining regulations according to water type.

A multivariate detector, e.g. mass spectrometer, in conjunction with chromatographic separation provides a data matrix for each peak formed by the abundances of K ions recorded at J times. Concatenating I of such matrices, a three-way tensor $\underline{\mathbf{X}}$ is obtained. This data tensor, or cube, contains all chemical information about the "same" peak in I chromatographic runs without simplifications. By means a multiway data analysis technique it is possible to decompose $\underline{\mathbf{X}}$ into as many factors as substances that are coeluting, providing the mass spectrum and the chromatographic profile of each of them.

Previous works [31–36] have demonstrated the usefulness of calibrations based on the PARAFAC decomposition of the three-way data obtained from chromatographic methods with detectors that give multivariate signals (DAD or MS). These works highlight the advantage of using the abundances recorded at all the ions selected (or the absorbancy spectrum) when quantifying or evaluating the method for identification in accordance with European Decision 2002/657/EC [37] which establishes the working criteria for methods used for detection and quantification of certain substances and residues thereof in animal products destined for human consumption. It has proved necessary to propose a multiway procedure of internal standardisation as well as procedures for the evaluation of figures of merit and other characteristics regarding the performance of the analytical method with the calibration based on the PARAFAC decomposition. Section 4 of Ref. [38] describes the stages of the procedure. It has also proven the usefulness of these calibrations for evaluation of robustness and in the optimisation of these methods [39] since the second-order property allows for the identification of the analyte of interest as a single factor independently of the change in instrumental factors or in the stages prior to extraction, even in the presence of unknown interferents which coelute with the analytes analysed.

The optimal performance of PARAFAC is under the trilinearity assumption. Trilinerity condition states that the spectral and chromatographic profiles should be the same in all samples, differing only in their sizes. In practice, the fact that the data are trilinear means that PARAFAC estimates must match up (except for scale factors) with the sample, chromatographic, and spectral profiles of the analytes. Therefore, it is possible to unequivocally identify each analyte using the spectral and chromatographic profiles, as the SANCO document states.

The PARAFAC model is greatly affected by deviations from the trilinear structure of the data. Slight changes in the retention time of an analyte between runs are usual in chromatography (generally greater in LC), and them the PARAFAC decomposition is no longer valid; a slightly different model, PARAFAC2, in which the chromatographic profile also depends on the i-th sample, is more accurate.

The PARAFAC2 model was proposed [40] in order to overcome this difficulty when modelling some deviations in chromatographic profiles. PARAFAC2 has the second order property if the correlation between the time profiles is the same in all samples, which is a weaker condition than the equality of chromatographic profiles imposed by the PARAFAC model. PARAFAC2 was used with GC/MS data in [38,39,41,42] and with LC/MS–MS in [43].

If the loss of trilinearity is important, then multivariate curve resolution (MCR) techniques are a useful alternative, because their

signal-related requirements are weaker than those demanded for PARAFAC or PARAFAC2. MCRALS has been applied on many occasions in analytical chemistry and its related fields [44,45], and it has been used to resolve coeluted compounds in hyphenated chromatography [38,46,47]. The major limitation of MCR in identifying and quantifying an analyte, as International Regulations Demand, is the presence of rotational ambiguities and non-unique solutions. The non-uniqueness problem can be alleviated or totally avoided in some cases through the intelligent use of the data structure and appropriate constraints. This problem is discussed in depth in [48].

When trilinearity is lost due to instrumental factors, e.g. column ageing, variability in mobile phase composition, this instrumental variability manifested as peak shifts could be eliminated. The alignment of one-dimensional chromatographic signals has been the subject of extensive research [49]. In addition, alignment of two-dimensional chromatographic signals such as the ones obtained an GC–MS or LC–MS steadily gains attention, because it is necessary to treat the information of different mass ratios separately in the warping function to ensure that compounds having the same m/z value and retention time are aligned to each other [50].

It is important to note that, among the conditions that must be fulfilled for the unequivocal identification of an analyte, shifts in its chromatographic peak must not exceed a certain value (0.5% for GC/MS or 2.5% in LC/MS in European regulations [1,37]) which ensures that deviations from trilinearity for this particular reason are not too large, and PARAFAC or PARAFAC2 can generally handle them.

This work completes the design of the analytical model proposing for the first time use of the second order property for the selection of the identifying ions which must be used in the analytical method based on GC–MS. This aspect is important because it depends on the instrumentation used and as is stated in Document SANCO/12495/2011 [1] "*the reference spectra for the analyte should be generated using the instruments and techniques employed for analysis of the samples. If major differences are evident between a published spectrum and that generated within the laboratory, the latter must be shown to be valid*".

The complete procedure will be put into practice to analyse three herbicides (Sz, Az and Pz) with Tz as IS. The procedure developed must fulfil the criteria established in the SANCO Document cited above which regulates the quality control procedures for pesticide residues analysis.

The first part of the work takes the data recorded in full scan mode (201 m/z ratios) and then uses: principal component analysis, PARAFAC and cluster analysis together with the specifications established in SANCO set out in Appendix A of Supplementary material (relative intensity, minimum number of ions per analyte to be analysed and maximum tolerance permitted for the identification) in order to select the m/z ions characteristic of each analyte.

The second part of the study focused on: (i) constructing a PARAFAC calibration with the data recorded in SIM (single ion monitoring) mode using the m/z ions selected previously, (ii) calculating the figures of merit and (iii) comparing the results with those obtained with a univariate calibration performed under the same conditions.

Finally, in the third section, by means a three-way matrix-matched calibration, two types of surface water (river and stream) and an underground water from a well were studied, using an SPE as a previous step. In addition to evaluating the recovery, this section also shows the importance of the second order property when an interferent coelutes.

The procedure designed in this work is general and allows one to completely develop an analytical method with multiway data in accordance with external specifications, from selection of identifying ions (m/z ratios), to quantifications and validation.

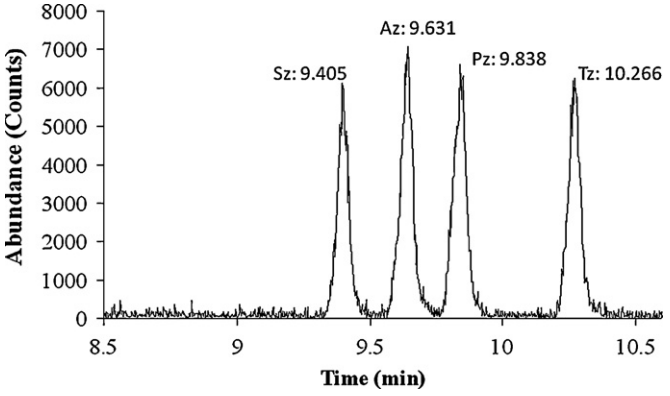

Fig. 1. Total ion chromatogram corresponding to the sample 4 of the design (Table 1) with the retention time of each analyte in the first chromatographic column. The sample contains 600 $\mu g\,L^{-1}$ of Sz and Az, 400 $\mu g\,L^{-1}$ of Pz and 500 $\mu g\,L^{-1}$ of Tz used as internal standard in the procedure to selection of variables recorded in full scan mode (201 m/z ions between 50 and 250).

2. Experimental

2.1. Chemicals and reagents

Triazines were obtained from Sigma–Aldrich (Madrid, Spain). Methanol and acetone (of gradient grade for HPLC) were purchased from Merck (Darmstadt, Germany). Deionised water was obtained by Milli-Q Gradient A10 water purification system of Millipore.

The cartridges used for the solid phase extraction (SPE) (Oasis HLB 200 mg, 6 mL) were purchased from Waters (Milford, MA, USA). In the conditioning, extraction, washing and elution phases, methanol, ethyl acetate, acetone and deionised water were used.

The water samples were collected from the Arlanzon River, from a section of the river in Castrillo del Val (a village near Burgos, Spain), from a stream and from a well belonging to the village of Olmillos de Sasamón (Burgos, Spain). These latter two water types were crop water, the former being surface water and the latter groundwater. In the three cases the water was collected in sterilised plastic containers, then frozen until analysis.

2.2. Standards and sample solutions

Stock standard solutions of each triazine at 100 mg L^{-1} were prepared in methanol and stored at low temperature (close to 4 °C) in amber glass containers for one year. The spiked solutions were prepared daily in acetone.

(i) Fifteen standard solutions were prepared by mixing the three triazines according to the central composite design of Table 1, thus having each of the analytes at five concentration levels (from 300 to 700 $\mu g\,L^{-1}$) and different levels of concentration for the other two. The concentration of the I.S. was fixed at 500 $\mu g\,L^{-1}$. The chromatogram of sample 4 in Table 1, is shown in Fig. 1. All chromatograms were recorded in the full scan mode (201 ions whose m/z ratios were comprised between 50 and 250 m/z).

(ii) In order to estimate some performance characteristics of the analytical method, in the second part of this work, a calibration was carried out with 10 external standard prepared in acetone at 5 concentration levels between 0 and 30 $\mu g\,L^{-1}$ (two replicates for each concentration level) of Sz, Az and Pz with the IS fixed at 10 $\mu g\,L^{-1}$. The chromatograms were recorded under the same analytical conditions and in SIM mode for the ions selected for each analyte with the procedure proposed in this paper. Another 5 samples at the same

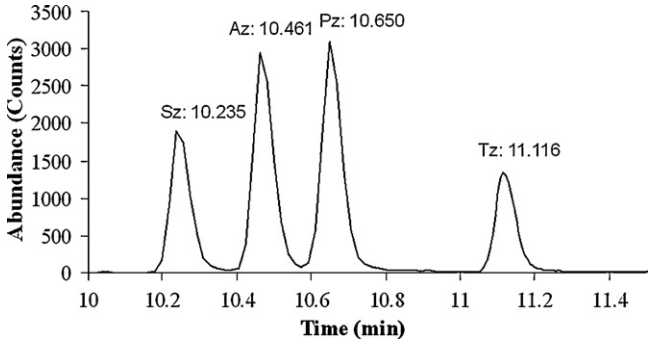

Fig. 2. Total ion chromatogram of a standard with 30 μg L^{-1} of Sz, Az and Pz and 10 μg L^{-1} de Tz recorded in SIM mode with the second column used as well as the retention time of each analyte.

concentration levels were used as test samples. Fig. 2 shows the chromatogram of a standard which contained 30 μg L^{-1} of each triazine and 10 μg L^{-1} of the IS. The difference in the retention times between the chromatograms in Figs. 1 and 2 is caused by the change of the chromatographic column which was necessary due to its ageing because six months passed between both experiments.

(iii) For the water analyses in the first study a solution of 100 mg L^{-1} was prepared, an intermediate solution of 1 mg L^{-1} of each analyte in acetone was used to made a solution of 50 μg L^{-1}, also in acetone, for each triazine. The external standard solutions used in the calibration were prepared the same day as the analysis. 33 external standard solutions were prepared in acetone, containing Sz, Az and Pz at 11 concentration levels between 0 and 5 μg L^{-1} (replicated three times) and 34 matrix matched standard solutions spiked with the triazines being studied, of which the first 7 were prepared in deionized water with concentrations between 0 and 6 μg L^{-1} of triazines and the remaining 27 in the two surface waters (stream and river) and the underground water (well) without enriching and enriched with concentrations of 2, 4 and 6 μg L^{-1} of the three analytes. In all the samples of these calibrations the Tz was fixed at a concentration of 2.7 μg L^{-1}.

(iv) The second study with the matrix matched solutions prepared in water was carried out to determine the detection capacity of the method, CCβ, at around 50 μg L^{-1}, beginning with the intermediate solution of 1 mg L^{-1} of each triazine in acetone taken from the same solution of 100 mg L^{-1} from the previous section. The enriched samples were prepared on the day of the analysis: 6 external standard with triazines at 3 concentration levels between 25 and 75 μg L^{-1} replicated twice and 12 samples of water enriched with the triazines being studied of which the first 6 were matrix matched standard solutions prepared in deionized water at 3 concentration levels for the three pesticides between 25 and 75 μg L^{-1} and the remaining 6 are three replicates of each sample in a surface water (stream) and one underground water (well) enriched with a concentration of 50 μg L^{-1} of the three triazines. In all the samples the concentration of IS was set at 25 μg L^{-1}.

2.3. Solid phase extraction (SPE)

Before carrying out the extraction process, the water samples were filtered and then enriched with the herbicides being studied and with the IS. For extraction the Oasis HLB 200 mg cartridges were used. The SPE procedure was carried out as in Ref. [51].

2.4. Chromatographic analysis

Analyses were performed with the Agilent 6890N gas chromatograph from Agilent Technologies, coupled with a simple quadrupole MS Agilent 5975 detector and an Agilent 7683 automatic injector. Separation was achieved with the J&W DB-5MS column from Agilent, J and W Scientific, Folsom, CA, USA, bonded-phase phenyl arylene (equivalent to 5% phenyl–methyl polyxiloxane), with a film thickness of 0.25 μm, and dimensions of 30 m × 0.25 mm I.D.

Injections were performed in the splitless mode with 9 min of solvent delay and using helium as carrier gas with a constant flow of 1.1 ml min^{-1}. The injector was kept at 225 °C, the ion source was 230 °C, the transfer line 250 °C and the quadrupole temperature was 150 °C. The oven temperature was programmed as follows: the initial temperature was set at 130 °C, increased from 130 °C to 160 °C at 10 °C min^{-1}, it was kept at 160 °C for 1 min and subsequently it raised to 180 °C at 3 °C min^{-1}, it was kept at 180 °C for 1.5 min. The oven equilibration time was set to 0.5 min. Sample injection volume was 2 μl. Analyses were carried out in the electron impact (EI) ionisation mode at 70 eV operating in full scan mode (201 ions whose m/z ratio was comprised between 50 and 250) and in SIM mode. The electron multiplier was fixed at 1329 V and the source vacuum at 10^{-5} Torr.

The full scan mode of the GC–MS was used to determine the compounds present in the first part of the paper, and the SIM mode was used to quantify them (second and third part of the paper). The dwell time per ion was 100 ms in all groups. Full scan analysis in mass spectrometry is commonly used as a data collection method in the prior exploration of pesticides. SIM recording of the same analytes provides greater sensitivity (as can be seen if one compares Figs. 1 and 2) but does not give any information about other substances which may be present in the sample or may even coelute with the analytes to be quantified [52] and some m/z ions are common.

For the quantification, four groups of ions were acquired in SIM mode. For Sz (group 1) the four ions, or m/z ratios, recorded were 68, 173, 201 and 203. For Az (group 2) the following ion fragments were selected: 68, 200, 202 and 215. For Pz (group 3) the following ion fragments were selected: 172, 214, 216 and 229. For Tz (group 4) 173, 214, 216 and 229 were selected as ions.

2.5. Software

The gas chromatography was controlled by GC ChemStation software and the data originated by the mass spectrometry were analysed using MSD ChemStation software and the NIST database [53].

The hierarchical clusterings were built by means of PARVUS [54]. PLS_Toolbox for Matlab version 6.5 [55] was employed for PCA and PARAFAC calculations. All regressions are validated with STATGRAPHICS [56]. A home-made program implemented over MATLAB, NWAYDET [57] was used to obtain the capability of detection (CCβ) and the decision limit (CCα).

3. Results and discussion

3.1. Selection of characteristic fragments

The experimental data, obtained from the samples prepared as in Table 1, were distributed in data tensors. To do this the chromatograms were fragmented around the retention time at which the abundance was maximum in each of the analytes thus obtaining four tensors of dimensions: $\underline{\mathbf{S}}$ (15 × 79 × 201), $\underline{\mathbf{A}}$ (15 × 66 × 201), $\underline{\mathbf{P}}$ (15 × 81 × 201) and $\underline{\mathbf{T}}$ (15 × 64 × 201). For each tensor: (i) the first dimension is the number of samples, that is, 15, (ii) the second

Table 1
Experimental matrix, with terbuthylazine (as I.S.) at 500 ($\mu g\,L^{-1}$).

Run	Experimental plan		
	Simazine ($\mu g\,L^{-1}$)	Atrazine ($\mu g\,L^{-1}$)	Propazine ($\mu g\,L^{-1}$)
1	400	400	400
2	600	400	400
3	400	600	400
4	600	600	400
5	400	400	600
6	600	400	600
7	400	600	600
8	600	600	600
9	300	500	500
10	700	500	500
11	500	300	500
12	500	700	500
13	500	500	300
14	500	500	700
15	500	500	500

dimension is the number of elution times (scans) used for each analyte. 76, 66, 81 and 64 scans were taken for the Sz, Az, Pz and Tz, respectively (the time for each scan was 0.00225 min), (iii) the third dimension is the number of ions (m/z) recorded of the mass spectrum, 201.

Using these data tensors, a sequential variable (m/z ratios) reduction was performed as shown in the scheme in Fig. 3 and is detailed in Sections 3.1.1–3.1.4.

3.1.1. Principal components analysis (PCA)

The objective is to eliminate redundant information and variability due to noise, selecting those m/z ions with highest *loading* from among the 201 loadings recorded. To do so, a matrix of data was constructed for each analyte, $\mathbf{S}_1$, $\mathbf{A}_1$, $\mathbf{P}_1$, and for the IS, $\mathbf{T}_1$ (the generic matrix $\mathbf{X}_1$ from step (i) in Fig. 3). The dimensions of these four matrices were 15×201, where 15 was the number of samples and 201 was the number of m/z ratios recorded in full scan mode. The matrix is made up by the abundance of each recorded m/z ion, for each sample, in the retention time (t_R) taken from de data recorded in full scan mode (see Fig. 1). In all the cases the start point was the tensor which contained all the data recorded in the experiment named generically $\underline{\mathbf{X}}$ in Fig. 3.

It is normal to apply some kind of data transformation prior to PCA to avoid the scale effect, but in this case all the variables are abundances recorded in the same scale and a pretreatment such as autoscaling would assign equal weight to the m/z ions with little abundance (which mainly have noise) as to those m/z ions with greater abundance. For this reason it was decided that no pretreatment should be applied. The number of principal components (CP) was obtained by minimising the root mean squares in cross validation (RMSCV) eliminating a sample each time ("leave one out method").

In the case of the Sz, the model with five principal components explained 96% of the variance of the matrix $\mathbf{S}_1$. The first component explained 90% (see Table S2) and the highest loading corresponds to the m/z ratio 201 which is the molecular ion and the base peak of the Sz. It was also noted that the rest of the variables (m/z ions) with high loading in this CP coincided with the most abundant m/z ratios. The other four CPs allowed for selection of less specific fragments of the simazine.

The data matrix, $\mathbf{A}_1$, of the Az also required 5 CPs and explained 98% of the variance of the predictors (Table S2); while in the case of the Pz almost the same percentage of explained variance of $\mathbf{P}_1$ was achieved but with 4 CPs (Table 4). Finally, in the matrix of data of $\mathbf{T}_1$ (Tz) 3 CPs explained almost 97.5% of the variance of which 96% was explained by only the first of them (Table S2).

The loadings of the 1st CP shown in Fig. 6, are linked with the spectral loadings obtained from the PARAFAC decomposition when the spectra are recorded in scan mode as can be seen by comparing them with the ions represented in Fig. S1 of Supplementary material. In addition, the fact that the data are not autoscaled nor centred means that the most significant m/z ratios of each analyte are found in this CP with higher loadings than the rest of the m/z ratios. The interpretation of the rest of the CPs is more difficult because the sign of the loadings did not remain constant, that is, each component either added or subtracted part of these loadings from the total of the decomposition performed to properly explain aspects of the spectrum not considered by the first component. In Table S2 are showed the m/z selected for each triazine.

As a consequence, 31 m/z ions were considered jointly for the four triazines. Thus it was possible to reduce the number of m/z ratios (variables) from 201 to 31 without losing relevant information contributed by the data since the most representative variables of the four analytes have been taken.

3.1.2. The relative intensities of the selected ions as a percentage of the intensity of the most abundant ion

In order to develop the second step of the procedure, step (ii) in Fig. 3, the chromatographic profile of each analyte was incorporated to the data matrix as second way. Thus, using the matrix of dimension (15×31) of each triazine, four new data tensors were constructed, $\underline{\mathbf{S}}_1$ ($15 \times 79 \times 31$), $\underline{\mathbf{A}}_1$ ($15 \times 66 \times 31$), $\underline{\mathbf{P}}_1$ ($15 \times 81 \times 31$) and $\underline{\mathbf{T}}_1$ ($15 \times 64 \times 31$), where the dimension of the first way was the number of samples, that of the second was the number of scans recorded for each analyte and that of the third, in this case, was the number of m/z ions selected by PCA in the previous stage. These four tensors are generically named as $\underline{\mathbf{X}}_1$ in Fig. 3.

Using the data of each triazine, a PARAFAC model was constructed imposing the restriction of non-negativity in the chromatographic mode. In the four cases only a single factor was needed. Thus, given the second order property of PARAFAC, there is only one chromatogram and one common spectrum for the 15 samples measured, independently of the solvent or matrix in which the substances are found.

The loadings were extracted from the spectral mode, Fig. S1(b), and the relative abundance of each m/z ratio was calculated in accordance with the directives of SANCO Document2007/3131 [58] point 79, reproduced in Supplementary material. Of the original 31 m/z ratios those which did not comply in at least one of the four pesticides studied with the requirement that the relative abundance of its loading with respect to the loading of the base peak be of at least 10% were eliminated. Table S3 shows the relative abundance of each m/z ion with respect to the base peak and the 20 m/z ratios greater than the 10% marked by SANCO are in bold.

3.1.3. Cluster analysis

After the stages described in Sections 3.1.1 and 3.1.2, 5 and 9 m/z ratios had been selected which fulfilled the requirements for the Tz and the rest of the triazines, respectively. As only four ratios were necessary [58] a further selection was needed, particularly for Sz, Az and Pz. The idea was to select for each triazine separately between 4 and 6 mass fragments the least similar to each other and rule out the most similar which gave redundant quantitative information. Cluster analysis of the variables was deemed suitable, thus constituting step (iii), in Fig. 3.

The ions with PARAFAC-loading greater than 10% of the PARAFAC-loading of the base peak in at least one of the target analytes are considered together because in this way the cluster analysis identifies those which are less similar including the ones that can identify another analyte. This is useful when the peaks have similar retention times and then, in SIM mode, they share the

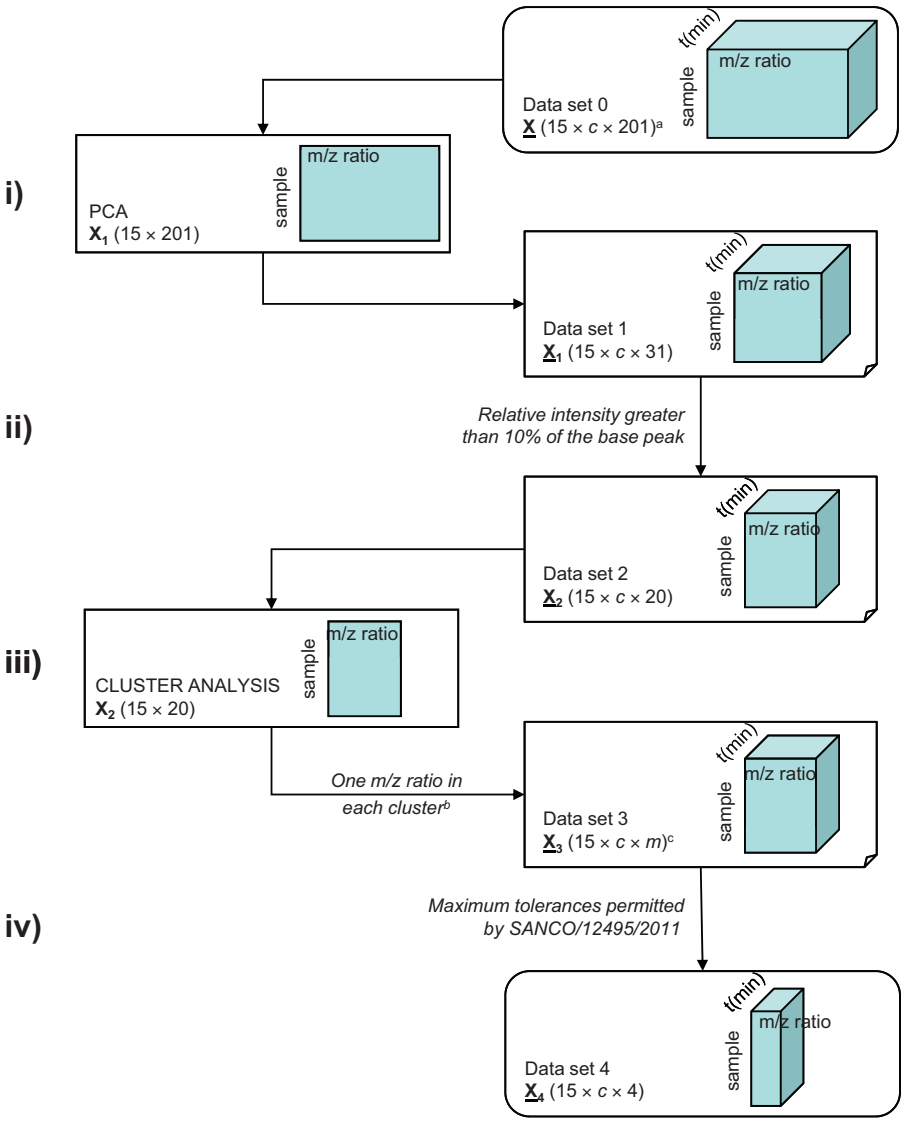

Fig. 3. Procedure diagram to variable selection (m/z ion ratios). (a) "c" are the scans recorded in the chromatographic mode, 79, 66, 81 and 64 for the Sz, Az, Pz and Tz, respectively. (b) A minimum of four ions should be selected from among those having a relative intensity higher than 10% of the base peak. (c) "m" is equal to 6 for the Sz, Az and Pz and equal to 5 for the Tz.

same window. So the data tensors of the triazines were formed by: the 15 samples with different concentrations, the scans of the elution times of the chromatograms of each analyte and the 20 m/z ratios $\underline{\mathbf{S}}_2$ ($15 \times 79 \times 20$), $\underline{\mathbf{A}}_2$ ($15 \times 66 \times 20$), $\underline{\mathbf{P}}_2$ ($15 \times 81 \times 20$) and $\underline{\mathbf{T}}_2$ ($15 \times 64 \times 20$). Using these cubes of data and the full abundance scan (t_R) new data matrices were constructed (named generically as $\mathbf{X}_2$ in Fig. 3) with dimensions 15×20 (number of samples × number of fragments of masses) for the Sz ($\mathbf{S}_2$), Az ($\mathbf{A}_2$) and Pz ($\mathbf{P}_2$). In the case of the Tz this was not necessary since only five of the 20 ions selected were fragments of this compound and its relative intensity with respect to the most abundant ion was higher than 10%, as can be seen in Table S3.

Those columns (m/z ions) with a value of zero for all the samples were eliminated from the matrices, such that the second dimension of the matrices $\mathbf{S}_2$, $\mathbf{A}_2$ and $\mathbf{P}_2$ was finally 15, 16 and 11, respectively. As a measure of similarity between the m/z ratios, the absolute value of the linear coefficient of correlation was used and complete linkage as agglomerative clustering method [59]. As is known, with this procedure the distance between groups of m/z ratios is equal to the smallest correlation (in absolute value) between pairs of variables.

The hierarchical clustering obtained is shown in Fig. 4. Setting the level of similarity at around 0.1 one obtains 5, 5 and 4 clusters for Sz, Az and Pz, respectively. Choosing one ion in each cluster are the identification points. Both in the case of the Az (Fig. 4b) and in the case of the Pz (Fig. 4c) the base peak and the molecular ion are in the same cluster but according to p. 80 of Appendix in Supplementary material both are selected although a priori they offer similar information because their abundances in the 15 samples are more correlated.

With this approach, the m/z ratios selected were: 68, 158, 173, 201 and 203 for the Sz; 68, 173, 200, 202, 215 and 217 for the Az and 69, 172, 214, 216 and 229 for the Pz. In the case of the Tz the five m/z ratios are: 172, 173, 214, 216 and 229 (which as already indicated come from the previous Section 3.1.2).

3.1.4. Threshold tolerances for the relative intensities of the detected ions

The last step, with respect to the criteria established in SANCO/12495/2011 (p. 80, Appendix of Supplementary material), was to test which of the m/z ratios selected were within the threshold tolerances imposed in the document. Analogous criteria are

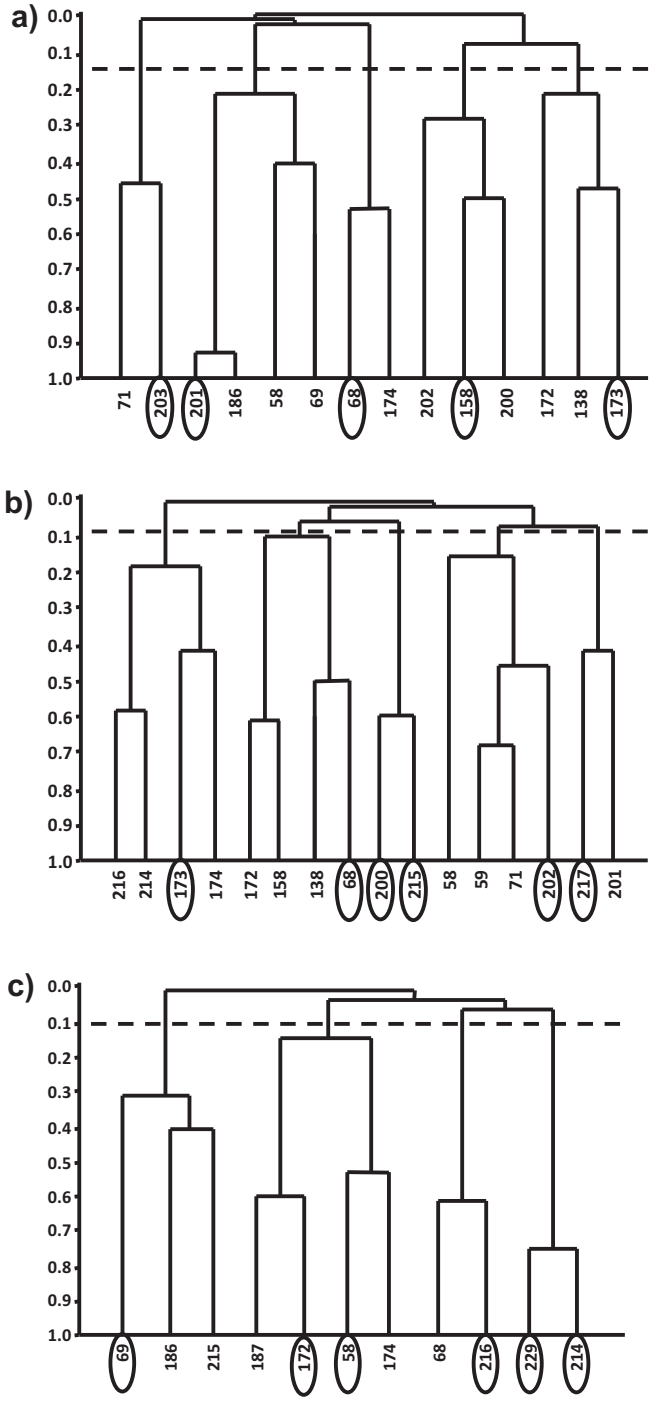

Fig. 4. Cluster of variables (mass fragments, m/z) for the three triazines. (a) Sz, (b) Az and (c) Pz. The similarity is represented in the ordinate axis from 0 (minimum similarity) to 1 (maximum similarity).

established in other regulated fields as in page 16 of European Union Decision 2002/657/EC [37]. This is step (iv) of the procedure which is shown in Fig. 3 associated with the generic tensor $\underline{\mathbf{X}}_3$.

To do this, using the variables selected in each cluster, new data tensors were formed: $\underline{\mathbf{S}}_3$ ($15 \times 79 \times 5$), $\underline{\mathbf{A}}_3$ ($15 \times 66 \times 6$), $\underline{\mathbf{P}}_3$ ($15 \times 81 \times 5$) and $\underline{\mathbf{T}}_3$ ($15 \times 64 \times 5$). The third way is formed by the ratios selected which are different for each analyte. The second way has the same scans initially recorded for each analyte.

The PARAFAC decomposition was carried out for each of the tensors, imposing the restriction of non-negativity in the chromatographic mode in all cases.

Only a single factor was required in all the PARAFAC models, which enabled identification of each analyte. Next, the *loadings* were extracted from the spectral mode of each of them and their relative abundance was determined with respect to the *loading* of the base peak in the spectral mode in each case. In order to verify that the spectral loading corresponds unequivocally with that of the corresponding triazine it is necessary to test that these relative abundances are within the tolerance threshold specified in SANCO. To do this it was also necessary to calculate the relative abundance of the ions in a standard sample containing 600, 600, 400 and 100 $\mu g\,L^{-1}$ of Sz, Az, Pz and Tz, respectively. The relative abundances of the standard sample allow one to determine the threshold tolerance permitted in each case according to the legislation cited earlier. Finally, it was checked what ions showed a relative abundance (calculated by means of the PARAFAC loadings) within the threshold tolerances and which did not.

Table 2 shows the relative intensities in terms of relative abundances and the tolerance intervals for the ions selected until that moment from the external standards. It also shows the loadings of the spectral mode of each PARAFAC model (one per analyte) and the relative abundance calculated from these loadings. It can be seen that for ions 158 in the Sz, 173 and 217 in the Az, 69 in the Pz and 172 in the Tz the relative abundance obtained was outside the permitted threshold tolerance. Therefore these m/z ions will not be used for the identification of the triazines.

After the selection process described, each triazine was shown to be characterised by four ions whose spectral loadings can be seen in Fig. S1(c). The m/z ratios selected with the process described, despite not coinciding in all the ions with those selected in the bibliography (Table S1) nor being the most frequent in the data base, are more suitable for the analysis according to the description on p. 76 of the SANCO document which advises taking note of the relations of the standards measured with the instrumentation used as opposed to those to be found in the library. What is more, the conditions demanded by SANCO are fulfilled, independently of the sample concentration. In other words, they were fulfilled for the 15 calibration standards simultaneously.

3.2. Calibration curves: univariate models and based on the PARAFAC decomposition

Before beginning the experimentation corresponding to this part of the work, it was necessary to change the chromatographic column, as already indicated in Section 2.2.(ii), which led to a variation in the retention times when comparing chromatograms in Figs. 1 and 2. In this case, both according Refs. [1,37], the relative retention times measured with the two columns must be controlled. In Table 3 it can be seen that the relative t_R with the new column were within the tolerance intervals calculated with the t_R obtained by the measurements taken with the old column. It can therefore be stated that the column change does not influence later results.

The 15 samples were prepared as described in Section 2.2 (ii). Ten of them were used as external standard calibration set and the remaining five as a test set. Two calibration models were applied and validated [56] using the same experimental data:

Univariate calibration: the steps followed in the univariate calibration were:

(i) Divide the original chromatograms into four intervals around the t_R of each substance (see Fig. 2). This allows one to analyse each analyte in isolation.
(ii) Select the base peak, or quantifying ion. In this work the m/z ratios selected were: 201, 200 and 214 for the Sz, Az and Pz, respectively; for the IS, the ion selected was 214 (see Table 2).

Table 2
Relative intensities of the detected ions, expressed as a percentage of the intensity of the most intense (abundant) ion, both for standard and for loadings of spectral mode of each PARAFAC model. Tolerances for relative ion intensities are shown as intervals permitted by Document SANCO/12495/2011 in fifth column.

	m/z	Standard			PARAFAC model	
		Abundance (counts)	Relative abundance (% of base peak)	Interval permitted by SANCO	Loadings (spectral mode)	Relative abundance (% of base peak)
Simazine	68	307	30.13	[25.61–34.65]	0.22	26.01
	158[b]	225	22.08	[18.79–25.39]	0.16	18.24
	173	426	41.81	[35.54–48.08]	0.33	38.65
	201[a]	1019	100.00	–	0.86	100.00
	203	297	29.15	[24.78–33.52]	0.26	30.13
Atrazine	68	229	17.26	[13.81–20.71]	0.13	15.74
	173[b]	413	31.12	[26.45–35.79]	0.19	23.65
	200[a]	1327	100.00	–	0.81	100.00
	202	461	34.74	[29.53–39.95]	0.25	30.90
	215	693	52.22	[47.00–57.45]	0.46	57.34
	217[b]	356	26.83	[22.80–30.85]	0.11	14.41
Propazine	69[b]	313	25.83	[21.95–29.70]	0.12	17.63
	172	673	55.53	[49.98–61.08]	0.39	55.58
	214[a]	1212	100.00	–	0.70	100.00
	216	357	29.46	[25.04–33.88]	0.22	31.65
	229	698	57.59	[51.83–63.35]	0.44	62.25
Terbuthylazine	172[b]	237	13.57	[10.86–16.28]	0.07	9.99
	173	606	34.69	[29.49–39.89]	0.32	36.81
	214[a]	1747	100.00	–	0.87	100.00
	216	529	30.28	[25.74–34.82]	0.28	31.75
	229	419	23.98	[20.38–27.58]	0.23	27.30

[a] Base peak (quantifier ion).
[b] Ion with relative abundance (% of base peak) non included into the interval permitted by Document SANCO/12495/2011.

Table 3
Ratios between the retention time of the analytes and retention time of internal standard and the intervals permitted by Document SANCO/12495/2011 for standards of Sz, Az and Pz with the old column and relatives retention times for the analytes with the new chromatographic column.

Analite	Old column (SCAN mode)			New column (SIM mode)	
	t_R (min)	Ratio of t_R (%) (t_R(analite)/t_R(Tz)) × 100	Interval permitted by SANCO[a]	t_R (min)	Ratio of t_R (%) (t_R(analite)/t_R(Tz)) × 100
Sz	9.405	91.6	[91.2–92.1]	10.235	92.1
Az	9.631	93.8	[93.3–94.3]	10.461	94.1
Pz	9.838	95.8	[95.4–96.3]	10.650	95.8
Tz (IS)	10.266	–	–	11.116	–

[a] The interval permitted by Document SANCO/12495/2011 is calculated as: ratio of t_R (%) ±0.5%.

(iii) Record the area for each m/z ratio and for all the concentrations.
(iv) Internal standardisation, dividing for each sample the area of each peak by the area recorded for ion 214 of the Tz (A_{Sz}/A_{Tz}, A_{Az}/A_{Tz} and A_{Pz}/A_{Tz}).
(v) Construct the univariate regression between the standardised areas and the true concentration of analyte.

Multiway model (PARAFAC decomposition): The details of the procedure to develop a PARAFAC calibration with chromatographic data can be consulted in Ref. [38]. Along general lines, the PARAFAC calibration is carried out in the following steps:

(i) Starting with the initial chromatograms, construct three data tensors, $\underline{\mathbf{S}}_4$, $\underline{\mathbf{A}}_4$, and $\underline{\mathbf{P}}_4$ of dimensions: $10 \times c \times 4$ where 10 is the number of calibration standards (sample mode), "c" equal to 14, 12 or 18, respectively is the number of scans recorded for each analyte, (chromatographic mode) and 4, the number of m/z ratios used to identify each analyte (spectral mode). A data cube was constructed for the IS, $\underline{\mathbf{T}}_4$, of dimensions: $10 \times 37 \times 4$. Working in this way one can make an independent study for each analyte.
(ii) Construct a PARAFAC model for each triazine and for the IS imposing the non-negativity restriction in the chromatographic mode. Choose the number of appropriate factors in each model and check, by applying statistics Q and T^2 the absence of outliers data. The four PARAFAC models have had a single factor.
(iii) Standardise each *loading* of the sample mode of each analytes, dividing these between the sample mode PARAFAC loadings of the IS.
(iv) Make a univariate regression between the standardised loadings of the sample mode and the true concentration of triazines.

The regressions constructed in this way provide in all cases, both for the univariate models and for the PARAFAC models, calculated concentrations which can be used to construct a new regression calculated concentration vs. true concentration, for each analyte. These regression models are shown in Table 4.

All the regressions were validated using the following test: significance of the regression, normality (Kolmogorov) and homocedasticity (Cochran and Barlett) of the residues, and lack of fit. In all cases the model proposed was correct at a significance level of 1% except in the univariate calibration of the propazine where the p-value for the Cochran test was below 0.01. Only in this case the residues of the data were not homocedastic. The Durbin–Watson autocorrelation test was also carried out, taking 1.4 as critical value. The p-values obtained for these tests with their corresponding null hypotheses are shown in Table S4 of Supplementary material.

Table 4
Performance characteristics determined for the analytical procedures based on univariate and PARAFAC: correlation coefficients, ρ; standard deviation of the regression c_{calc} vs. c_{true}, s_{yx} ($\mu g\,L^{-1}$); mean of the absolute value of the relative errors (%) in calibration ε_{cal} and prediction, ε_{pred}; limit of decision, CCα ($\mu g\,L^{-1}$); and capability of detection, CCβ ($\mu g\,L^{-1}$); $\alpha = \beta = 0.05$.

	Simazina		Atrazina		Propazina	
	Univariate	PARAFAC	Univariate	PARAFAC	Univariate	PARAFAC
Intercept (p-value)[a]	5×10^{-4} (0.999)	-4×10^{-5} (0.99)	1×10^{-4} (0.999)	-1×10^{-4} (0.999)	-9×10^{-4} (0.998)	-1.2×10^{-3} (0.998)
Slope (p-value)[b]	0.995 (0.877)	0.999 (0.06)	1.001 (0.971)	1.0003 (0.991)	0.998 (0.944)	1.008 (0.808)
ρ	0.996	0.997	0.997	0.997	0.997	0.996
s_{yx}	1.005	0.916	0.972	0.913	0.970	1.102
ε_{cal} ($n = 8$)	6.29	5.78	7.24	4.73	6.16	6.66
ε_{pred} ($n = 4$)	5.96	7.28	5.46	7.06	6.08	6.28
CCα	2.10	1.90	2.01	1.89	2.02	2.27
CCβ	4.08	3.68	3.92	3.68	3.92	4.41

[a] Null hypothesis: the intercept is zero.
[b] Null hypothesis: the slope is one.

To determine the trueness of the regression models, it was checked that at a significance level of 0.05, the confidence intervals found for their slopes and their independent terms included both one and zero, respectively. Their p-values can be seen in Table 4.

3.2.1. Limit of decision and detection capability

Despite the fact that the SANCO document does not specifically require it, these two figures of merit were calculated since the SANCO document makes several references as to how the results are expressed as in Decision 2002/657/CE and the ISO itself proposes in ISO-18143 [60]. The advantage of this method of expressing the results is that it guarantees the probability of error of false positives and false negatives. To determine the limit of decision, CCα, and the detection capability, CCβ [37,61,62], the probability of false positive and false negatives was set at 0.05. In the case of first and higher-order signals modelled by two and superior-order calibrations, the capability of detection can also be determined through both probabilities α (false positive) and β (false negative). The details of generalisation to multi-way calibrations can be seen in Refs. [57,63]. This procedure can be used with signals of any order and any calibration method since the capability of detection can be determined using the linear regression.

The CCα found for the simazine, atrazine and propazine were of 2.10, 2.01 and 2.02 $\mu g\,L^{-1}$, respectively and the CCβ of 4.08, 3.92 and 3.92 $\mu g\,L^{-1}$ when univariate calibration models were used for their determination. By calculating these values using the PARAFAC models the results obtained for CCα were 1.90, 1.89 and 2.27 $\mu g\,L^{-1}$ and for CCβ 3.69, 3.68 and 4.41 $\mu g\,L^{-1}$ for Sz, Az and Pz, respectively. Given that the samples were not pretreated in order to preconcentrate the sample, the limits found are adequate.

With respect to the mean of absolute values of relative errors in calibration, values of 6.29, 7.24 and 6.16%, for Sz, Az and Pz were obtained respectively, when using univariate models and 5.89, 4.73 and 6.66% for each triazine, if the PARAFAC models are used (with $n = 8$ in each case).

3.2.2. Prediction samples

The prediction samples were analysed the same day as the external calibration standards. The test set was used to study the prediction capacity of the calibration models constructed earlier (Section 3.2).

In order to apply the multiway calibration, prediction samples were added to the data tensor already constructed with the external calibration standards. The PARAFAC calibration was performed, imposing the non-negativity restriction in the chromatographic mode with the new data tensors with dimensions $15 \times c \times 4$, where the dimension of the sample mode is 15 (10 standards and 5 samples), "c" the dimension of the chromatographic mode (14, 12, 18, or, 37 for Sz, Az, Pz, or Tz respectively), and 4 the dimension of the spectral mode. A single factor was necessary in each case. Q and T^2 statistics were used to check that the test samples were similar to those of the calibration and no anomalous data were detected.

With both the univariate and the multiway approaches, the identification of the analytes was carried out through retention times and by confirmation by mass spectrometry.

In the first case, the tolerance interval has to be applied for the relative retention time. Table 5 shows these intervals for the univariate external calibration standards and for the *loadings* of the chromatographic mode in the PARAFAC decomposition. The relative retention times of the analytes in the prediction samples fulfilled this requirement both in the univariate model (abundance in the corresponding ion) and in the PARAFAC model (loadings of the chromatographic mode). The PARAFAC decomposition provides a single chromatographic profile common to all the samples which intervene in the tensor. Table 5 also gives the relative retention times calculated for the four problem samples which contain triazine both for the univariate model and for the PARAFAC model.

In the second, one must follow the guidelines of the SANCO Document (pp. 74–75), but bearing in mind that the relative abundances of the confirmation ions with respect to the base peak, calculated for identification by MS, depend on the concentration of the standard sample. Table 6 shows the relative abundances and the tolerance intervals permitted by the SANCO/12495/2011 for the ions recorded of the three triazines at the four concentration levels (5, 10, 20 and 30 $\mu g\,L^{-1}$) of the univariate approach and for the *loadings* of the spectral mode of the PARAFAC decomposition. Both were constructed using the calibration samples. While with the univariate approach one had four possible reference spectra for each triazine, one for each non-null concentration, with the PARAFAC decomposition a single spectral profile was obtained for each analyte, common to all the calibration samples. Table 7 shows the results obtained both in univariate and in multiway calibration.

The univariate approach determined that 50, 86, 89 and 67% of the m/z ratios recorded (12 per analyte, 36 in total) showed a relative abundance within the permitted interval when the standard used was the calibration sample of 5, 10, 20 and 30 $\mu g\,L^{-1}$ of triazine, respectively. It must be borne in mind that all the samples had concentrations above CCα and CCβ, but the identification by means of mass spectrum failed in a high percentage of cases depending on the concentration level of the sample with which the tolerance interval was calculated for the different identifying ratios chosen (m/z). This problem, the discrepancy between the quantitative detection (samples with analyte concentration above the decision limit) and the qualitative detection (mass spectrum which does not fulfil the tolerances with respect to a reference tolerance) which in practice leads to false negatives, has already been pointed out as a drawback when using univariate approach [64,65].

Table 5
Ratios between the retention time of the analytes and retention time of internal standard (t_R ratio (%)) and the intervals permitted by Document SANCO/12495/2011 for standards of Sz, Az and Pz. All values have been calculated at all levels of concentration with the retention times of base peak (univariate) and with the loadings of PARAFAC (multivariate).

			Tz (m/z = 214)	Sz (m/z = 201)	Az (m/z = 200)	Pz (m/z = 214)
5 μg L^{-1}	Calibration standard	t_R	11.12	10.24	10.47	10.66
		t_R ratio (%)[a]		92.14	94.14	95.84
		Interval permitted		[91.68–92.60]	[93.67–94.62]	[95.37–96.32]
	Test sample	t_R ratio (%)[a]		92.16	94.14	95.82
10 μg L^{-1}	Calibration standard	t_R	11.12	10.25	10.47	10.66
		t_R ratio (%)[a]		92.15	94.14	95.85
		Interval permitted		[91.69–92.61]	[93.67–94.61]	[95.37–96.33]
	Test sample	t_R ratio (%)[a]		92.17	94.15	95.85
20 μg L^{-1}	Calibration standard	t_R	11.12	10.25	10.47	10.66
		t_R ratio (%)[a]		92.15	94.13	95.82
		Interval permitted		[91.69–92.61]	[93.66–94.60]	[95.34–96.30]
	Test sample	t_R ratio (%)[a]		92.18	94.14	95.83
30 μg L^{-1}	Calibration standard	t_R	11.12	10.24	10.47	10.65
		t_R ratio (%)[a]		92.15	94.14	95.84
		Interval permitted		[91.69–92.61]	[93.66–94.61]	[95.36–96.31]
	Test sample	t_R ratio (%)[a]		92.17	94.16	95.85
Loadings[b]	Calibration standard	t_R	11.11	10.22	10.48	10.65
		t_R ratio (%)[c]		92.03	94.34	95.87
		Interval permitted		[91.57–92.49]	[93.87–94.81]	[95.39–96.35]
	Test sample	t_R ratio (%)[c]		92.19	94.08	95.78

[a] being t_R the retention time of Sz, Az, or, Pz respectively.
[b] Loadings of the chromatographic profile of the PARAFAC model: the dimensions of tensors $\underline{\mathbf{X}}$ were $(10 \times c \times 4)$, where c was equal to 14, 12 or 18 for Sz, Az or Pz, respectively (Section 3.2: multiway model).
[c] Ratio calculated with the PARAFAC-loadings.

For the multiway model, the ratio was calculated, as a percentage, between the loading of the quantifying ion and the loading of the base peak considered for each triazine, and checked whether the abundance was within the tolerance intervals calculated previously (Table 6). All the m/z ratios recorded fulfilled this requirement (a total of 9 m/z ratios, 3 for each substance). In contrast to what happened with the univariate analysis, the use of PARAFAC eliminated the contradiction, as has already been shown in Ref. [43].

As can be seen in Table 4, with the univariate calibration, the mean of the errors in prediction was 5.96, 5.46 and 6.08% for the Sz, Az and Pz, respectively, while with PARAFAC it was 7.28, 7.06 and 6.28% for the Sz, Az and Pz, respectively.

3.3. Water samples

The method proposed for the analysis of the triazines was applied to 9 sample sets shown in Table 8. As can be seen the samples differ in the matrix: the water samples were enriched with the triazines (including the I.S.) before the solid phase extraction procedure:

(i) $\underline{\mathbf{A}}$ and $\underline{\mathbf{F}}$ are data tensors which were constructed using the external standards of triazines prepared in acetone as described in Section 2.2 (iii) and (iv), respectively.
(ii) Tensors $\underline{\mathbf{B}}$ and $\underline{\mathbf{G}}$ were formed using the data recorded for the matrix matched standards prepared in deionised water. These samples were submitted to the solid phase extraction (SPE) process described in Section 2.3 and were later injected in the GC–MS. The two tensors differ in their concentration range.
(iii) $\underline{\mathbf{C}}$, $\underline{\mathbf{D}}$, $\underline{\mathbf{E}}$, $\underline{\mathbf{H}}$ and $\underline{\mathbf{I}}$ correspond to the data tensors constructed using the data recorded for samples of the two types of surface water (stream: $\underline{\mathbf{C}}$ and $\underline{\mathbf{H}}$, and river: $\underline{\mathbf{E}}$) and the underground water (well: $\underline{\mathbf{D}}$ and $\underline{\mathbf{I}}$) which were first submitted to SPE and then analysed by CG–MS. These samples were used for the validation of the analytical method proposed.

That is, in all pesticide determinations a "matrix-matched calibration" as defined in SANCO document is applied.

3.3.1. Figures of merit

The data recorded in the analysis of the external standards prepared in acetone (tensor $\underline{\mathbf{A}}$) and matrix matched standards prepared in deionised water (tensor $\underline{\mathbf{B}}$), were used to construct two multiway regression models following the steps described in Section 3.2. The four triazines required PARAFAC models with a single factor. Using statistics Q and T^2, it was checked that there were no outliers data. The spectral loadings were also extracted in order to calculate the relative abundance of each m/z ion acquired and to find the permitted tolerance intervals for each of the ions. Both the relative abundances and the intervals calculated were important since when later the sample problems of the different types of water are introduced it will be necessary to check whether the relative abundances calculated for their m/z ratios are found to be within these intervals in order to be able to unequivocally identify them. Finally the loadings of the sample profile of the analytes were standardised by means of the loading from IS and a univariate regression was made between the standardised loadings of the sample profile and the true concentration of triazines in the samples. The calibration of each triazine with the sample profile (loadings of the PARAFAC decomposition for tensor $\underline{\mathbf{A}}$) standardised with the sample profile of the IS allowed one to obtain the figures of merit when working with external standard solutions prepared in acetone as solvent.

Additionally, the joint decomposition of tensors $\underline{\mathbf{A}}$ and $\underline{\mathbf{B}}$ permitted the determination in each data tensor of the common factor related with the triazines. With sample profile extracted for this factor, the recovery of the method for each triazine was calculated as the relation in % between the slope of the regression *standardised sample loadings* vs. *true concentration*, obtained with the matrix matched solutions prepared in deionised water ($\underline{\mathbf{B}}$) and the slope of the regression *standardised sample loadings* vs. *added concentration* of the external standard solutions in acetone ($\underline{\mathbf{A}}$). This procedure could be applied since the independent term of the regressions was significantly equal to zero in both calibrations, with the exception of the calibration of the atrazine whose standard deviation from the regression is very small and the signification have no sense.

The results for the figures of merit of these calibrations are shown in Table 9. Analysis of this table allows one to affirm

Table 6
Abundances, ratios of abundance between the ions and base peak and the intervals permitted by Document SANCO/12495/2011 for standards of Sz, Az and Pz. All values have been calculated at all levels of concentration with the maximum peak abundances (univariate) and with the loadings of PARAFAC (multivariate).

Sz		*m/z* ratio			
		201[a]	68	173	203
5 μg L^{-1}	Abundance	139	57	61	38
	Relative abundance (%)		41.01	43.88	27.34
	Interval permitted		[34.86–47.16]	[37.30–50.47]	[23.24–31.44]
10 μg L^{-1}	Abundance	170	56	73	58
	Relative abundance (%)		32.94	42.94	34.12
	Interval permitted		[28.00–37.88]	[36.50–49.38]	[29.00–39.24]
20 μg L^{-1}	Abundance	416	128	180	135
	Relative abundance (%)		30.77	43.27	32.45
	Interval permitted		[26.15–35.38]	[36.78–49.76]	[27.58–37.32]
30 μg L^{-1}	Abundance	700	287	338	210
	Relative abundance (%)		41.00	48.29	30.00
	Interval permitted		[34.85–47.15]	[41.04–55.53]	[25.50–34.50]
PARAFAC	Loading [b]	0.84	0.30	0.38	0.27
	Relative loading (%)		35.37	44.99	32.28
	Interval permitted		[30.06–40.67]	[38.24–51.73]	[27.44–37.12]
Az		*m/z* ratio			
		200[a]	68	202	215
5 μg L^{-1}	Abundance	182	47	64	96
	Relative abundance (%)		25.82	35.16	52.75
	Interval permitted		[21.95–29.70]	[29.89–40.44]	[47.47–58.02]
10 μg L^{-1}	Abundance	268	59	82	121
	Relative abundance (%)		22.01	30.60	45.15
	Interval permitted		[18.71–25.32]	[26.01–35.19]	[38.38–51.92]
20 μg L^{-1}	Abundance	638	146	200	271
	Relative abundance (%)		22.88	31.35	42.48
	Interval permitted		[19.45–26.32]	[26.65–36.05]	[36.11–48.85]
30 μg L^{-1}	Abundance	1082	260	335	501
	Relative abundance (%)		24.03	30.96	46.30
	Interval permitted	0.83	[20.43–27.63]	[26.32–35.61]	[39.36–53.25]
PARAFAC	Loading[b]		0.18	0.27	0.45
	Relative loading (%)		21.69	32.53	54.22
	Interval permitted		[18.43–24.94]	[27.65–37.41]	[48.80–59.64]
Pz		*m/z* ratio			
		214[a]	172	216	229
5 μg L^{-1}	Abundance	165	106	57	98
	Relative abundance (%)		64.24	34.55	59.39
	Interval permitted		[57.82–70.67]	[29.36–39.73]	[53.45–65.33]
10 μg L^{-1}	Abundance	232	149	72	123
	Relative abundance (%)		64.22	31.03	53.02
	Interval permitted		[57.80–70.65]	[26.38–35.69]	[47.72–58.32]
20 μg L^{-1}	Abundance	617	390	186	336
	Relative abundance (%)		63.21	30.15	54.46
	Interval permitted		[56.86–69.53]	[25.62–34.67]	[49.01–59.90]
30 μg L^{-1}	Abundance	1085	641	325	575
	Relative abundance (%)		59.08	29.95	53.00
	Interval permitted		[53.17–64.99]	[25.46–34.45]	[47.70–58.29]
PARAFAC	Loading[b]	0.75	0.45	0.24	0.43
	Relative loading (%)		60.00	32.00	57.33
	Interval permitted		[54.00–66.00]	[27.20–36.80]	[51.60–63.07]

[a] Base peak of each analyte.
[b] The dimensions of tensors $\underline{\mathbf{X}}$ were ($10 \times c \times 4$), where c was equal to 14, 12 or 18 for Sz, Az or Pz, respectively (Section 3.2: multiway model).

that the methodology employed permits not only unequivocal identification of the pesticides but also allows for their quantification at levels from 0.17 to 1.37 μg L^{-1} for those cases where the probability of false positive is set at 0.05 and values between 0.35 and 2.6 μg L^{-1} when the probability of false negative is also fixed at 0.05. These values prove similar than values found in the bibliography.

3.3.2. Surface and underground water samples

To the data tensor corresponding to each triazine made up until this moment with $\underline{\mathbf{A}}$ and $\underline{\mathbf{B}}$ were joined tensors $\underline{\mathbf{C}}$, $\underline{\mathbf{D}}$ and $\underline{\mathbf{E}}$ corresponding to the three types of natural water (Table 8). Fig. 5 shows the tensor of dimensions ($67 \times 12 \times 4$) corresponding to the Sz.

In the case of the Az and Pz a single factor was necessary to identify the corresponding triazines, but in the case of the Sz the

Table 7
Ratios of abundance between the ions and base peak for the test samples with Sz, Az and Pz with the maximum peak abundances (univariate). Ratios of the spectral loadings obtained in the PARAFAC models with 15 samples for the three analytes.

Simazina (Sz)		*m/z* ratio			
		201[e]	68	173	203
5 μg L^{-1}	Abundance	163	44	70	59
	Relative abundance (%)		26.99[a,d]	42.94	36.20[a,d]
10 μg L^{-1}	Abundance	247	75	105	82
	Relative abundance (%)		30.36	42.51	33.20[a]
20 μg L^{-1}	Abundance	586	175	240	196
	Relative abundance (%)		29.86[a,d]	40.96[d]	33.45[a]
30 μg L^{-1}	Abundance	677	216	297	234
	Relative abundance (%)		31.91[a,b,d]	43.87	34.56[a,d]
PARAFAC	Loading	0.837	0.293	0.376	0.268
	Relative loading (%)		34.95	44.97	31.99
Atrazina (Az)		*m/z* ratio			
		200[e]	68	202	215
5 μg L^{-1}	Abundance	206	20	72	122
	Relative abundance (%)		9.71[a,b,c,d]	34.95	59.22[a,b,c,d]
10 μg L^{-1}	Abundance	371	79	121	165
	Relative abundance (%)		21.29[a]	32.61	44.47
20 μg L^{-1}	Abundance	852	186	240	368
	Relative abundance (%)		21.83[a]	28.17[a]	43.19[a]
30 μg L^{-1}	Abundance	990	184	335	583
	Relative abundance (%)		18.59[a,b,c,d]	33.84	58.89[a,b,c,d]
PARAFAC	Loading	0.836	0.176	0.269	0.444
	Relative loading (%)		21.02	32.12	53.11
Propazina (Pz)		*m/z* ratio			
		214[e]	172	216	229
5 μg L^{-1}	Abundance	232	144	73	120
	Relative abundance (%)		62.07	31.47	51.72[a]
10 μg L^{-1}	Abundance	346	224	105	189
	Relative abundance (%)		64.74	30.35	54.62
20 μg L^{-1}	Abundance	776	514	234	403
	Relative abundance (%)		66.24[d]	30.15	51.93[a]
30 μg L^{-1}	Abundance	915	618	288	486
	Relative abundance (%)		67.54[d]	31.48	53.11[a]
PARAFAC	Loading	0.750	0.450	0.239	0.431
	Relative loading (%)		60.00	31.86	57.46

[a] Non-compliant sample when the permitted interval has been calculated with standard reference sample at: 5 μg L^{-1}.
[b] Non-compliant sample when the permitted interval has been calculated with standard reference sample at: 10 μg L^{-1}.
[c] Non-compliant sample when the permitted interval has been calculated with standard reference sample at: 20 μg L^{-1}.
[d] Non-compliant sample when the permitted interval has been calculated with standard reference sample at: 30 μg L^{-1}.
The permitted interval at each level is shown in Table 5.
[e] Base peak of each analyte.

Table 8
Characteristics of samples used to obtain some figures of merit. **A**, **B**, **C**, **D** and **E** at null concentration and **F**, **G**, **H** and **I** at 50 μg L^{-1}.

Dataset (dimension)	Analytical matrix	Concentration levels (μg L^{-1})
A (33 × 12 × 4)	Acetone	0.0, 0.5, 1.0, 1.5, 2.0, 2.4, 3.0, 3.6, 4.0, 4.5, 5.0
B (7 × 12 × 4)	Deionized water	0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0
C (9 × 12 × 4)	Stream water	0.0, 2.0, 4.0, 6.0
D (9 × 12 × 4)	Well water	0.0, 2.0, 4.0, 6.0
E (9 × 12 × 4)	River water	0.0, 2.0, 4.0, 6.0
F (6 × 12 × 4)	Acetone	25.0, 50.0, 75.0
G (6 × 12 × 4)	Deionized water	25.0, 50.0, 75.0
H (3 × 12 × 4)	Stream water	50.0
I (3 × 12 × 4)	Well water	50.0

decomposition needed two factors to correctly explain this analyte. Fig. 6 shows the three profiles extracted in the PARAFAC model for this analyte. The first factor (blue) corresponded to the Sz in agreement with the chromatographic and spectral profile; a second factor (green) presented practically null loadings in the sample profile for the external standards (prepared in acetone), while for the samples which had been submitted to the SPE process, these were non-null, their effect being greater in the samples of natural water than in the deionised water. As regards the spectral profile, this factor only showed two of the *m/z* ratios recorded: ion 68 and 173 (Fig. 6d).

A more thorough study of the chromatograms recorded selecting only *m/z* ratio 68 for the different samples shows the presence of an interferent whose retention time was close to that of the simazine. Fig. 7 shows three chromatograms of each of the different types of sample analysed. It can be seen how in the case of

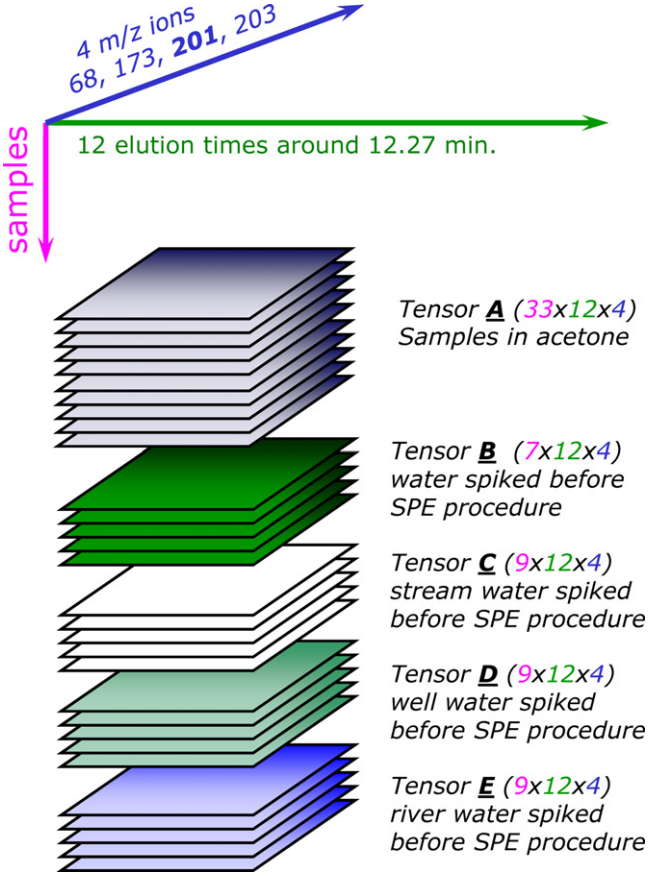

Fig. 5. Construction of the data tensor joining tensors **A**, **B**, **C**, **D** and **E** used to determine the figures of merit of the calibration model proposed for the Sz.

external standards prepared in acetone and whose detection was carried out with no later prior SPE extraction Fig. 7a, the Sz signal, recorded for ion 68, is a single peak, but when the same signal is analysed in the same times for the matrix matched solutions prepared in deionised water and stream water and reconstituted in acetone/ethyl acetate after the solid phase extraction process, the Sz peak had an anomaly in the tail Fig. 7b and c. In water, the triazines are subject to processes of degradation such as photolysis, oxidation, hydrolysis and biodegradation which lead to the loss of alkyl groups. Ref. [16] indicates that one of the products of this transformation is desisopropilatrazine whose molecular mass is 173 and one of its characteristic ions is 68. It is highly likely that the factor extracted by PARAFAC is this structure, although it is not possible to unequivocally identify it with only two ions. As has been shown, the presence of this coeluent does not modify the identification or the quantification of the simazine. In general, using jointly the external standards in acetone and the matrix-matched ones, the second order property of the PARAFAC decomposition allows one, as in this case, to quantitatively determine the simazine even in samples which have an interferent which coelutes with it.

3.3.3. Identification

When applying the univariate methodology the criterion of relative tolerances with a standard sample of 1, 3 and 5 $\mu g\,L^{-1}$ failed in many cases (false negatives), above all when compared with the lowest concentration levels. Globally, false negatives of 73%, 98% and 51% were obtained for the Sz, Az and Pz, respectively when the data were analysed using the univariate methodology. That is, of the 21 test samples of different types of water (stream, well and river) spiked with concentrations of each triazine above the limit decision, many of them did not fulfil the tolerance criteria imposed for their determination. On the other hand, when the spectral profile, common to all the acetone samples and obtained with the PARAFAC decomposition was used as standard, the identification

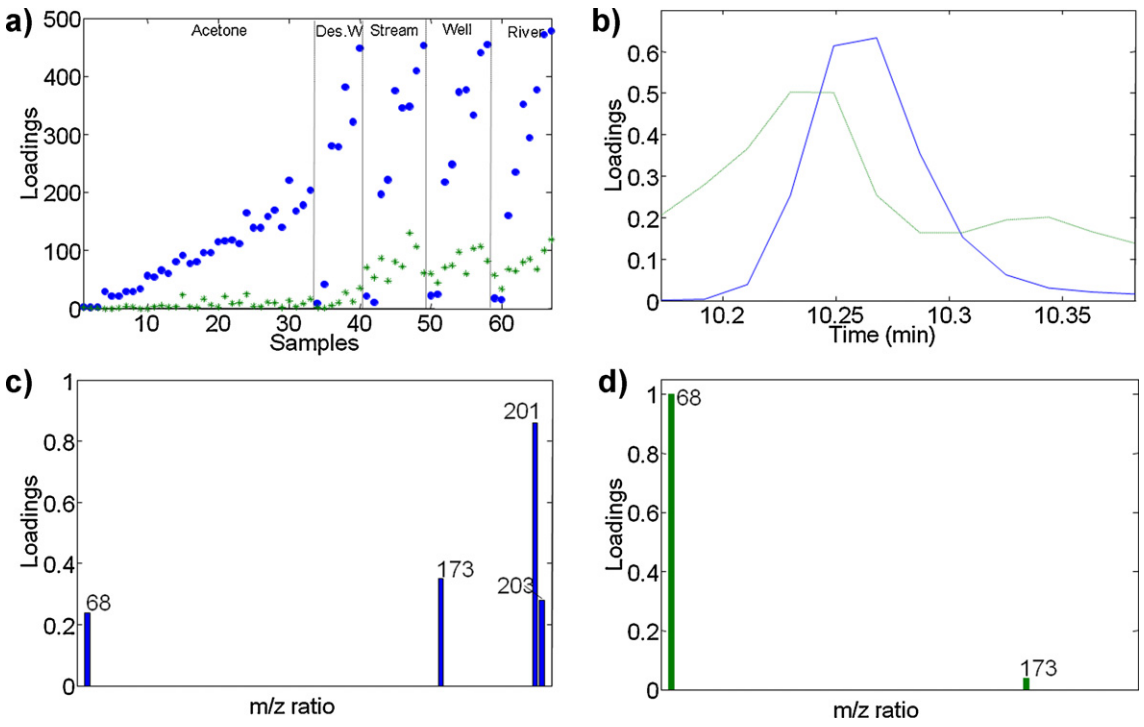

Fig. 6. Two factors PARAFAC decomposition of tensor shown in Fig. 5. (a) Loadings of the sample mode without internal standardisation. (b) Loadings of the chromatographic mode. The first factor represented in blue with circles and a continuous line is related to the Sz while the second, represented in green by asterisks and a dotted line is related to an interference which gives a significant signal at the ratio *m*/*z* 68. Graphs (c) and (d) show the spectral loadings of the Sz and the interferent, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

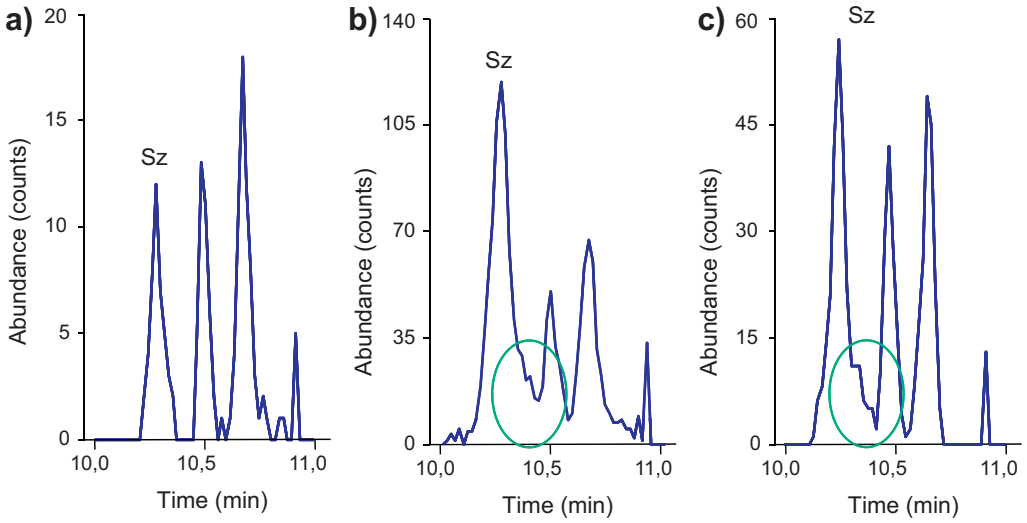

Fig. 7. Chromatograms recorded setting the m/z ion at 68 for the samples enriched with 2 μg L^{-1} of Sz in (a) acetone, (b) deionised water and (c) stream water.

criteria were fulfilled for the three traizines in all their m/z qualifier ions.

3.3.4. Figures of merit in 50 μg L^{-1}

To end the study, the behaviour of these analytes was studied in water at higher concentrations since despite the fact that Sz and Az are regulated substances, Pz is not and can be found at higher concentrations.

New PARAFAC models were calculated for the three triazines with tensors **F**, **G**, **H** and **I** shown in Table 8. As in the preceding case, a external calibration is built with loadings of cube **F** and other matrix-matched calibration with loadings of cube **G**. The calibration slopes obtained from these models were validated and the trueness of the models was evaluated by means of regressions of calculated concentration vs. true concentration, setting the significance level at 5%. After that, the triazines in water samples of steam and well spiked with 50 μg L^{-1} (its data are the tensors **H** and **I**) are quantified. These water samples are those already analysed in Section 3.3.2 and they were free of triazines. The mean of absolute value of the relative errors was situated between 1 and 6% in calibration and between 7 and 10% in prediction except for the Sz when it is determined using the matrix matched standard prepared in deionised water subjected to SPE, in which case the errors rose to 19% in calibration and 27% in prediction. The determination of the concentration of Az and Pz in water samples does not differ significantly when this is calculated using the external calibration or using a matrix matched calibration. On the other hand, in the case of the Sz, if one wants to obtain a good estimation of the spiked concentration in the sample problems this must be calculated using the external standard calibration. The recoveries thus achieved were: 84, 89 and 88% for the Sz, Az and Pz, respectively in stream samples and 94, 102 and 106% for the well samples.

3.4. Calibration maintenance

The potential application of the proposed methodology in routine analysis for screening and/or quantification of pesticide residues require ensuring the validity of the calibration. In that context, rather than fit a calibration model for each batch of samples, is it more practical to have a strategy to maintain an initial calibration model. With this purpose, the SANCO document states the need to assure the calibration performance and the testing for replacement of standards. Therefore, the use of periodic control samples, SQC, is mandatory.

Like in the determination with water samples, Section 3.3, the procedure would consist of adding to the tensor made up by the calibration samples, **A**, the matrix **C** of the control sample. This matrix contains the target analytes in known concentrations. Thus, in the PARAFAC decomposition of the new **A** + **C** tensor, the factor corresponding to the target analyte which is present in all the samples would be obtained. The regression between the sample loadings and the concentration of the calibration samples allows estimation of the concentration that corresponds to the loading of the con-

Table 9
Characteristic parameters and figures of merit of the calibration slopes standardised samples loadings *vs* added concentration obtained by the analytical method based on the PARAFAC decomposition of the three triazines: intercept (p-value) and slope; correlation coefficient, ρ; standard deviation of the regression, s_{yx} (μg L^{-1}); mean recovery (%); mean of the absolute value of the relative errors in calibration, ε_{cal}, and prediction, ε_{pred}; decision limit (CCα) and capability of detection (CCβ) for a probability of false positive, α, and false negative, β, both set at 0.05, respectively.

	Simazine		Atrazine		Propazine	
	Acetone	Water	Acetone	Water	Acetone	Water
Intercept[a] (p-value)	0.002 (0.45)	0.008 (0.56)	0.014 (0.0001)	0.018 (0.41)	0.022 (0.01)	0.008 (0.71)
Slope	0.068	0.036	0.111	0.052	0.149	0.056
ρ	0.998	0.974	0.999	0.947	0.989	0.978
s_{yx}	0.006	0.020	0.009	0.029	0.026	0.028
Recovery (%)	53.09		46.85		37.45	
ε_{cal}	4.87	8.98	4.10	8.27	4.80	5.69
ε_{pred}	13.88	10.69	19.05	18.28	8.55	13.75
CCα (μg L^{-1}) at x_0 = 0 μg L^{-1}	0.17	1.34	0.15	1.37	0.31	1.23
CCβ (μg L^{-1}) at x_0 = 0 μg L^{-1}	0.35	2.58	0.30	2.63	0.63	2.36

[a] Null hypothesis: intercept is equal to 0.

trol sample. This concentration must be significantly equal to the known concentration in SQC.

Small changes on the measurement system will appear as variations in the spectral profile and/or in the chromatographic profile, depending on whether tensor $\underline{\mathbf{A}}$ or $\underline{\mathbf{A}+\mathbf{C}}$ is analysed. Of course, the standardised spectral loadings must be into the tolerance intervals and the position of the maximum of the chromatographic profile will comply with the requirements about the relative retention time. More important changes will cause the loss of trilinearity in the data or the appearance of one or several new factors related to the control sample, similarly to the coeluting substance with Sz whose detection is described in Section 3.3.2.

In this way, the characteristics of the signal (spectral and chromatographic) are monitored as well as the sensitivity of the calibration model trough the concentration obtained for the control sample.

Another possible alternative will be to project matrix **C** in the space spanned by the PARAFAC decomposition of tensor $\underline{\mathbf{A}}$. However, previous studies [57,66] indicate that the results are better when analyzing tensor $\underline{\mathbf{A}+\mathbf{C}}$.

4. Conclusions

The procedure proposed in the first part of this paper allows one to select, from the *m*/*z* ratios recorded in full scan mode, those which, complying with the criteria mentioned in the SANCO document, are able to unequivocally identify the three triazines. Methodologically the designed procedure performs as expected. However, authors are working on its simplification to make its use easier for those analysts who are not familiar with multiway techniques.

A calibration curve is made relating the standardised loadings extracted from the sample mode in the PARAFAC decomposition for each triazine to the true concentration of each of them. In this way one can use the second order property of this decomposition. The mean of absolute value of the relative errors in calibration are close to 5% and the values obtained for CCα and CCβ are around 2 $\mu g\,L^{-1}$ and 3 $\mu g\,L^{-1}$, respectively. It can be seen that when one compares the results, although they do not differ greatly between the univariate process and the multiway based on PARAFAC (mainly due to the absence of both coeluents and of a matrix effect), the results obtained are better for both CCα and CCβ when all the information available about the chromatograms and the analyte spectra is analysed jointly. The same conclusion is reached in the case of the errors in calibration. Furthermore, analysis of the data by means of a PARAFAC decomposition is simple and quick.

The working criteria require calculation of the tolerance for all the retention times and all the *m*/*z* ratios at all the concentration levels of the samples used in calibration and in prediction (more than 20 and 70, respectively in this work), while the PARAFAC decomposition, thanks to its second order property which enables the unequivocal identification of an analyte, provides a single chromatogram and a single common spectrum for all the samples measured and it is only necessary to check that they comply with a retention time and three tolerances for the *m*/*z* ratios for each triazine. The procedure based on PARAFAC avoids the contradiction between the quantitative detection (values above the decision limit) and the qualitative detection (identification by means of mass spectra) observed when the calibration is univariate and is not based on all the ions used in the identification.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2012.05.017.

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