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Monitoring of pesticides in drinking and related waters in NE Spain with a multiresidue SPE-GC–MS method including an estimation of the uncertainty of the analytical results

Jordi Quintana, Isabel Martí, Francesc Ventura*

Department of Organic Analytical Chemistry, AGBAR. Societat General d'Aigües de Barcelona, Pg Sant Joan 39-43, 08009 Barcelona, Spain

Abstract

A new method, developed in an EC project (SMT4-CT96-2142) for the determination of 22 pesticides in drinking and related waters, has been used to analyze source and drinking water samples in the area of Barcelona (NE Spain). The procedure includes solid-phase extraction of water and subsequent analysis by GC–MS using few selected ions (SIR) in order to increase their sensitivity. The method was subjected to intra and interlaboratory tests and met the requirements of the EC Directive in terms of accuracy, precision and detection limit (0.025 μg/l range in water samples). A detailed analysis of the uncertainty sources of this method is included, which allows to estimate expanded uncertainties in the 10–20% range. The dominant sources of uncertainty are the solid-phase extraction procedure and the chromatographic quantification. Two triazine compounds, simazine and atrazine, are the main pesticides detected in wells of the Llobregat river and in water of the Ter river, respectively.

Keywords: Water analysis; Solid-phase extraction; Uncertainty analysis; Pesticides

1. Introduction

Since the late decades, concern about the contamination of water sources has risen due to the increasing number of pesticides detected. Regulations for drinking water are required in order to limit human risks and environmental pollution. These regulations are well defined in North America, depending on the toxicity level of each compound, and Europe [1], setting at 0.1 μg/l of pesticide concentration for a single pesticide compound and 0.5 μg/l for the sum of all pesticides in water samples. While in the USA, EPA methods exist in order to standardize methods around the states [2,3], in Europe no specific method has been given [4]. Consequently, it becomes necessary to provide control laboratories with analytical methods allowing the monitoring of pesticide residues at this trace level, with basic performance data in agreement with the drinking water EC Directive 98/83 requirements. In this context, the overall goal of the European project 'Optimization and Evaluation of Multiresidue Methods for Priority Pesticides in Drinking and Related Waters' (SMT4-CT96-2142) was to develop a new multiresidue method in order to support the implementation of the Drinking Water Directive. Seven laboratories from five different countries provided control laboratories with documented and validated multiresidue methods allowing the monitoring of a broad range of priority pesticides. The developed methods were subjected to

*Corresponding author. Tel.: +34-93-3423-651; fax: +34-93-3423-666.

E-mail address: fventura@agbar.es (F. Ventura).

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intra and interlaboratory tests in order to evaluate if the method met the requirements of the EC Directive in terms of accuracy, precision and detection limit of 25% related to parametric value for the common interest pesticide list (see Table 1). This list included fourteen herbicides, two metabolites and six insecticides largely used, like triazines, organochlorinated and organophosphorus compounds [5].

This paper shows one of these methods using a styrene divinylbenzene copolymer SPE to pre-treat the sample and further analysis of the extract by gas chromatography using mass spectrometry (GC–MS) with electron impact ionization (EI) as a detector. SPE is particularly suited for the isolation of organic micropollutants from water and has now become the method of choice in order to carry out simultaneously the extraction and concentration of many pesticides and metabolites in aqueous samples [6–8]. The most widely used sorbents are C_8 and C_{18} chemically bonded to silica, carbon black and polymeric resins [6]. But in the last few years, new styrene–divinylbenzene packing materials have been developed allowing greater π–π interactions between analytes and the sorbent [9–11]. Otherwise, mass spectrometry is recognized as a highly sensitive and specific technique in environmental organic analysis [12,13] and the most common technique used by laboratories involved in pesticide analysis. The sensitivity of this technique can be increased in two orders of magnitude (ng/l level) by using a few selected and characteristic ions for each compound and should be used to obtain the required EC directive concentration level. Moreover, multiresidue methods to cover all the main groups of pesticides are desired and require the universality of sample pretreatment procedure and the same conditions for the chromatographic separations [14]. For example, Benfenati et al. [15] reported the simultaneous analysis by GC–MS with EI of 50 pesticides of several groups in water samples, Fillion et al. [16] a multiresidue method for the determination of residues of 251 pesticides in fruits and vegetables by GC–MS and HPLC or analysis of 51 polar pesticides at trace levels in aqueous samples by using SPE and thermospray LC–MS [17].

The aim of this work is to determine the occurrence of the selected pesticides in river, groundwater and treated water of Barcelona (NE Spain) and its metropolitan area by using this SPE procedure [5].

In addition, an estimation of the uncertainty of the analytical determinations was carried out in order to determine the critical stages of the analytical process and to introduce high quality analytical performance. The ‘bottom-up’ method is used in order to estimate each individual uncertainties for every single step of the measurement process and obtain the combined standard uncertainty from the sum of each contribution. Few papers describe this step of analytical measurement in pesticide analysis. Thus, Lisinger et al. [18] measured the uncertainty for the determination of triazines by HPLC using the ‘top-down’ method from analysis validation data. This analysis showed an expanded uncertainty close to 10% when

Table 1
Acquisition data for GC–MS analysis of the multicomponent solution of 22 pesticides by using SIR mode, two selected ions for each compound and five acquisition windows

Compound	Retention time (min)	*m/z*
Dichlobenil	6.92	171, 173
DIA	13.92	158, 173
DEA	14.32	172, 174
Trifluralin	14.78	264, 306
Dimethoate	16.95	87, 125
Simazine	17.64	186, 201
Atrazine	18.02	200, 215
Lindane	18.25	181, 219
Propazine	18.40	214, 229
Terbutylazine	18.99	214, 229
Pirimicarb	21.10	166, 238
Metribuzin	22.15	144, 198
Alachlor	22.65	160, 188
Terbutryn	24.06	226, 241
Ethofumesate	24.24	161, 286
Metolachlor	24.65	162, 238
Chlorpyriphos-ethyl	24.84	314, 316
Cyanazine	25.10	225, 240
Metazachlor	26.48	132, 209
Pendimethalin	26.48	162, 252
α-endosulfan	28.32	195, 241
β-endosulfan	30.95	195, 241

Five acquisition windows in SIR mode: 4 to 16 min, 16 to 20.5 min, 20.5 to 24.5 min, 24.5 to 27 min and 27 to 32 min. Ions 188 and 189 are selected for anthracene-d10, used as an internal standard, in the second acquisition window (retention time of 19.3 min). Chromatographic conditions described in the Materials and methods section.

concentrations in the range of 0.2–0.6 μg/l are analyzed and close to 25% for the lowest concentration studied (0.05 μg/l).

2. Materials and methods

2.1. Chemicals and materials

Dichlobenil (99%), desisopropylatrazine (DIA) (99.5%), desethylatrazine (DEA) (99%), trifluralin (99.6%), dimethoate (99.8%), simazine (99.2%), atrazine (99%), lindane (99.9%), propazine (99.6%), terbutylazine (99%), pirimicarb (99.5%), metribuzin (99.7%), alachlor (99.7%), terbutryn (98%), ethofumesate (99.5%), metolachlor (97.8%), chlorpyriphos-ethyl (99%), cyanazine (99%), metazachlor (99%), pendimethalin (98.3%), α-endosulfan (99.8%) and β-endosulfan (98.9%) were purchased from Riedel de Haën (Seelze, Germany). Anthracene-d10 used as an internal standard was purchased from Supelco (Bellefonte, PA). All reference materials certified were used as received and with an individual certificate of analysis. All materials are toxic and were handled in accordance with the most current material safety data sheets. Methanol and ethyl acetate of organic residue analysis grade were supplied from J.T. Baker (Deventer, The Netherlands), whereas isooctane of pesticide residue analysis grade were supplied from Carlo Erba (Rodano, Italy). HPLC grade water was obtained from a Maxima water purification system (USF-Elga, High Wycombe Bucks, UK).

Stock standard solutions of each compound (1 mg/ml) were prepared by weight in ethyl acetate, except simazine (0.2 mg/ml). A mixed standard solution of all pesticides was prepared at 10 ng/μl in ethyl acetate. Eight multicomponent calibration solutions (from 0.025 to 0.5 ng/μl) were prepared from the previous solution by dilution with isooctane. An anthracene-d10 solution (5 ng/μl) was prepared in isooctane and 20 μl of this solution were added to 0.5 ml of extracts or calibration solutions as internal standard in chromatographic quantification. All solutions were stored frozen in the dark at −20°C until use.

2.2. Geographical situation

To examine the feasibility of the method, water samples from the water distribution system of Barcelona area and from its sources were analyzed. The Llobregat and Ter rivers, situated in the south and north of Barcelona, respectively, supply Barcelona's drinking water (45% and 55%, respectively). These rivers have different water qualities due, among other factors, to the presence of salt mining and densely industrialized and inhabited areas along the banks of the Llobregat river. This salinity gives a characteristic salty taste to the water treated at the Sant Joan Despí water treatment plant (WTP1) and Abrera (WTP2). Otherwise, the Ter river, with better raw water quality, is situated in an important agricultural area. The Ter river is treated in a third WTP (WTP3) and transported to Barcelona through a pipeline. Groundwater from the aquifer of the Llobregat river is also used for human consumption, collected from wells close to WTP1 and situated in an agricultural area. This groundwater can be incorporated to WTP1 in the water treatment process in drought periods. The different consumption needs of the treated water in the city often require the blending of these waters.

A third river close to Barcelona (Besòs river) has been unused for human consumption since the 1950s due to high contamination levels. Due to the improvement of the raw water quality in this river by corrective measures taken, groundwater from the aquifer of the Besòs river was also analyzed to evaluate its quality to serve as drinking water for east-Barcelona surrounding cities.

2.3. Sampling

All samples were collected during the year 2000. Different sources of water determine different water qualities, depending on which areas of Barcelona were sampled. Roughly, east Barcelona and surroundings receive treated water from the Ter river (WTP3) and west Barcelona and surroundings receive treated water from the Llobregat river (WTP1 and WTP2). River water and treated water from WTP1 and WTP2 were also analyzed. Groundwater from the Llobregat river aquifer was collected from wells placed near WTP1. Groundwater from the

Besòs river aquifer was collected from an old and unused Besòs WTP.

Samples were collected in Pyrex borosilicate amber glass containers (2 l). Surface water samples with high levels of suspended solids or turbidity were filtered through cellulose filters (0.45 μm pore size, under vacuum) in order to avoid subsequent clogging of the solid support used for extraction. Filtration could be avoided in the case of clean waters such as drinking water or groundwater. Sodium thiosulphate (2 m*M* of sample) must be added to drinking water or other chlorinated water in order to avoid the degradation of pesticides before SPE procedure. Sample preservation was accomplished by storing the bottles at 4°C after sampling. Extraction is carried out as soon as possible, within 7 days after collection.

2.4. Sampling preparation procedure

SPE extraction columns Bakerbond spe™ were used and purchased from J.T. Baker (Deventer, The Netherlands). Each column contains 6 ml disposable extraction cartridges packed with 200 mg styrene divinylbenzene copolymer (SDB). To extract and concentrate all samples, an Autotrace SPE workstation and TurboVap LV evaporator are used (Zymark, Hopkinton, Mass.).

A 500 ml volume of water, in which 5 ml of methanol had been added, was passed over the conditioned sorbent (3 ml of ethyl acetate, 3 ml of methanol and 6 ml of water) at a flow-rate of 5 ml/min. The sorbent was afterwards dried under nitrogen for 20 min. Elution was performed by soaking the cartridge with 2.5 ml of ethyl acetate at a flow-rate of 0.8 ml/min, eluted with a second portion of 2.5 ml of ethyl acetate and collected in a glass conical vessel containing 0.5 ml of isooctane. The eluted was then dried under a gentle stream of nitrogen to 0.45 ml. The volume was precisely adjusted to 0.5 ml of isooctane. Twenty μl of internal standard solution (5 ng/μl) was added to the extract and stored frozen at −4°C until used for analysis.

2.5. GC–MS determination

All analyses were carried out with a GC 8000 series capillary gas chromatograph coupled to a MD800 mass spectrometer (Fisons Instruments, Manchester, UK). Separations were conducted on a DB-5 MS fused-silica capillary column, 30 m×0.25 mm I.D.×0.25 μm film thickness (J&W Scientific, Folsom, CA), with helium as a carrier gas, at a flow of 1.5 ml/min. The column was held at 85°C ramped at 10°C/min to 150°C, then up to 160°C at 1°C/min and finally ramped at 5°C/min to 250°C and held for 5 min. A volume of 1 μl of sample was injected on a splitless injector by using an AS 800 autosampler (CE Instruments, Rodano, Italy). The injector temperature was set at 270°C. The mass spectrometer was operated in the EI mode. The parameters were set at the following values: An electron energy of 70 eV and a filament emission current of 200 μA. The interface and ion source temperatures were maintained at 270 and 200°C, respectively. The instrument was operated in SIR mode, in five acquisition windows at 0.08 scan/min each one, and two selected ions for each compound for identification and quantification were monitored. These ions were selected following the criteria of highest relative abundance, characteristic fragment ions and no interferences with the nearby peaks. Table 1 summarizes all acquisition data. MassLab version 1.4 software (Finnigan, Manchester, UK) was used for data acquisition.

Pesticides in real samples are identified when the following criteria are accomplished: The chromatographic peaks of both ions from the unknown and the standard must coincide at the same retention time and the ratio between the two selected ions must be the same in both the real sample and calibration mix (with a tolerance of ±15%).

Linear plot (peak area versus concentration) was constructed by using the calibration mix. The pesticide is quantified by interpolation of the sum of two selected ions peak areas into the linear plot, whereas the anthracene-d10 area is used to correct instrumental variations. Results were corrected by using recovery values.

3. Results and discussion

3.1. Performance of the SPE

The performance of the method was evaluated according to AOAC guidelines [19]. This method

should, at least, be able to measure concentrations equal to EC Directive's parametric value with trueness, precision and a limit of detection of 25% related to parametric value (0.1 μg/l in water samples). These criteria were interpreted such as limit of detection 0.025 μg/l in water samples, trueness expressed as a recovery, which has to be between 75% and 125%, and precision expressed as a repeatability and reproducibility of the method, <12.5% and <25%, respectively. The results of the method performance are summarized in Table 2.

3.1.1. Linearity

Linearity was evaluated by the calculation of an eight-point linear plot, based on linear regression and the correlation coefficient '*r*', which should be >0.995. These solutions were prepared from a mixed standard solution of all pesticides (10 ng/μl in ethyl acetate). Otherwise, linearity range was determined from the linear plot, where $(y_{i+1} - y_i)/(x_{i+1} - x_i)$ must be constant into the linear range with an interval of confidence ±10%. In the extract (1000 times more concentrated than water samples), most pesticides had a linear range from 0.015 to 0.04 to 0.5 ng/μl and an average correlation coefficient above or equal to 0.997. Both pendimethalin and metribuzin had a linear range from 0.15 to 0.5 ng/μl; DIA, dimethoate metribuzine, cyanazine and pendimethalin had correlation coefficients between 0.992 and 0.996.

3.1.2. Detection limits

Detection limits were calculated as three times the standard deviation of seven spiked samples of commercially available bottled water at a concentration of 0.025 μg/l. Most compounds were under 25% of parametric value of EC Directive (<0.025 μg/l). Only pendimethalin and metribuzine had a detection limit in water samples higher than the value required (0.062 and 0.035 μg/l, respectively).

Table 2
Intralaboratory exercise results for spiked samples (0.1 μg/l) of commercially available bottled water with a multicomponent solution mix analyzed by all laboratories involved in the EC project SMT4-CT96-2142

Pesticide	'r'[a]	Linear range[a] (ng/μl)	LOD[a] (μg/l)	Repeat.[a] (%) (*n*=4)	Reprod.[b] (%) (*n*=4)	Recovery[a] (%)
Dichlobenil	0.998	0.025–0.500	0.009	9.6	12.8	76
DIA	0.995	0.025–0.500	0.008	7.3	14.8	96
DEA	0.997	0.025–0.500	0.009	7.8	13.5	95
Trifluralin	0.997	0.035–0.500	0.013	5.5	10.6	73
Dimethoate	0.992	0.025–0.500	0.019	5.2	16	69
Simazine	0.998	0.025–0.500	0.008	6.7	12.8	98
Atrazine	0.998	0.025–0.500	0.008	6.8	13.8	86
Propazine	0.999	0.015–0.500	0.005	6.6	15.6	83
Lindane	0.998	0.030–0.500	0.009	8.7	7.8	47
Terbutylazine	0.998	0.030–0.500	0.010	6.0	10.1	84
Pirimicarb	0.998	0.010–0.500	0.011	7.5	12.6	84
Metribuzine	0.996	0.150–0.500	0.035	8.5	20.5	80
Alachlor	0.998	0.040–0.500	0.012	6.5	15.6	73
Terbutryn	0.997	0.040–0.500	0.013	8.0	5.5	104
Ethofumesate	0.999	0.040–0.500	0.013	5.1	11.8	95
Metolachlor	0.998	0.035–0.500	0.012	5.2	9.5	102
Chlorpyriphos-ethyl	0.998	0.045–0.500	0.013	8.8	8.0	78
Cyanazine	0.994	0.040–0.500	0.013	7.4	17.4	131
Metazachlor	0.997	0.040–0.500	0.014	6.4	16.9	76
Pendimethalin	0.993	0.150–0.500	0.060	7.3	7.1	68
α-endosulfan	0.999	0.040–0.500	0.011	7.5	14.4	78
β-endosulfan	0.998	0.030–0.500	0.011	7.4	16.4	93

Detection limits were calculated as three times the standard deviation of seven spiked samples at a concentration of 0.025 μg/l. Repeatability, reproducibility and recovery were evaluated in terms of relative standard deviation of four spiked samples of 0.1 μg/l.

[a] The linear plot data (linear range in the extract), LOD (in water samples), repeatability and recoveries correspond to our laboratory data results in the intralaboratory exercise.

[b] The reproducibility data are the average data for all laboratories in the intralaboratory exercise.

3.1.3. Precision

Repeatability and reproducibility were evaluated in terms of relative standard deviation of four spiked water samples of 0.1 μg/l analyzed by all laboratories involved in that project. Each laboratory's data were used to repeatability and all laboratories data were used to reproducibility. Relative standard deviation for all compounds were under the value required (repeatability $<12.5\%$ and reproducibility $<25\%$).

3.1.4. Trueness

Trueness is expressed as a recovery, which has to be between 75% and 125%. Choice of SPE was previously discussed by Pichon [20]. Nine different phases had been selected and tested (C_{18}, polymeric and graphitized carbon black type). Finally, the styrene divinylbenzene copolymer was the best choice. Most compounds had a recovery between 75% and 125%. Lindane (47%), trifluralin (73%), dimethoate (69%), pendimethalin (68%) and cyanazine (131%) presented recovery values out from this interval, and this was overcame by applying recovery values to the final chromatographic concentration. The high-performance of SDB is attributed to the aromatic structure of the polymer, which can interact with aromatic analytes via $\pi-\pi$ interactions [9]. Thus, low recovery of lindane and dimethoate may be attributed to the absence of an aromatic ring in its molecular structure. Similar recoveries by using SDB sorbents, specially for triazines and their degradation products [6], have been reported for the other compounds.

3.1.5. Matrix effect study

In addition, a second interlaboratory exercise was performed with 15 more European laboratories, by using the same analytical protocol as previously described, in order to establish quality parameters of the method in bottled and treated water. The interlaboratory results were comparable and confirmed those of the intralaboratory study. No significant interferences were detected and the method was suitable for all listed compounds. When river water was analyzed, organic natural substances can give interfering and unwanted peaks that cause irreproducible results; this problem is avoided by using the SDB sorbent which, owing to the small pore size of the sorbent, excludes humic substances at neutral pH, which indicates a type of size exclusion during sorption [21].

Therefore, this method is reliable for most compounds. Only pendimethalin and metribuzine have limits of detection and linear range outside of the Directive.

3.2. Estimation of uncertainty

The estimation of the uncertainty of analytical results is one of the main focuses of interest in the field of measurement chemistry. Both the traceability and the degree of confidence, given as parameters that define the quality of an analytical result, must be demonstrated.

The estimation of the uncertainty of analytical results is mandatory for laboratories accredited under EN45001 requirements (CEN/CENELEC, 1989). Detailed analysis of uncertainty sources can show critical stages of analytical method where uncertainty should be reduced. The main approaches to calculate uncertainty, which have been proposed to date, are the 'bottom-up' and 'top-down' methods. The former assesses each individual uncertainty for every single step of the measurement process, where the combined standard uncertainty results from the sum of each contribution. The 'bottom-up' method was proposed by ISO in order to quantifying uncertainty in physical measurements and was subsequently adapted by EURACHEM [22]. On the other hand, the 'top-down' approach uses validation data and data from proficiency testing schemes to estimate the uncertainty of the method. A disadvantage of the latter method compared with the 'bottom-up' method is that no information about the variation of uncertainty is available and no corrective actions can be performed on critical stages of analysis.

For most purposes, in analytical chemistry, an expanded uncertainty (U) should be used. The expanded uncertainty provides an interval within which the value of the mesurand is believed to lie with higher level of confidence. U is obtained by multiplying the combined standard uncertainty (u) by a coverage factor (k). The choice of this factor is based on the level of confidence desired.

Although our method was validated and, therefore, data were available to use the 'top-down' method,

the most suitable procedure to quantify uncertainty was to evaluate each uncertainty component separately by using the 'bottom-up' approach method, and to do it as a relative uncertainty in order to cover all the concentrations range. Thus, the general procedure used in combining individual components was the following: (a) to prepare a detailed quantitative model of experimental procedure; (b) to assess the standard uncertainties associated with the individual input parameters, and (c) to combine them by using the law of propagation of uncertainties.

The general relationship between the combined standard uncertainty $u_c(y)$ of a value y and the uncertainty of the independent parameters $x_1, \ldots x_n$ on which it depends is:

$$u_c^2[y(x_1, \ldots x_n)] = \Sigma_i \, (\partial y/\partial x_i)^2 u(x_i)^2 \quad (1)$$

where $y(x_1, \ldots x_n)$ is a function of several parameters $x_1, \ldots x_n$ and $\partial y/\partial x_i$ is the partial differential of y with respect to x_i. $u(x_i)$ can be calculated using this last procedure or may also be evaluated directly by repetitive experiments measuring n times x_i; uncertainty can be estimated from standard deviation of the mean:

$$u(x_i) = S \cdot \omega/n^{1/2} \quad (2)$$

where S is the standard deviation of n measurements and ω is a correction factor that differs from the unity only when n is <10 [22]. This evaluation of uncertainty is particularly valuable where no reliable mathematical description of the relationship exists between $x_1, \ldots x_n$.

Otherwise, for models involving only a product or quotient of statistically independent variables, the combined standard uncertainty $u_c(y)$, where $y = f(x_1, \ldots x_n)$, is given by:

$$u_c^2[y(x_1, \ldots x_n)] = [u(y)/y]^2 = [u(x_1)/x_1]^2 + \ldots + [u(x_n)/x_n]^2 \quad (3)$$

where $[u(x_i)/x_i]$ are the uncertainties in the parameters, expressed as relative standard uncertainties.

The function used to calculate the final concentration in our analytical method is:

$$\text{Concentration } (C) = [(m/V_i) \cdot V_e \cdot rec]/V_s \quad (4)$$

where: C is the final concentration of a pesticide expressed in μg/l, m is the chromatographic amount (in μg) quantified by applying the regression equation to the chromatographic area, V_i is the volume injected into the GC (in μl), V_e is the volume of the extract (in μl) resulting from the SPE procedure, V_s is the volume of the water sample (in l) and *rec* is the recovery obtained for each compound.

The combined relative standard uncertainty corresponding to Eq. (4) is:

$$u_c^2(C) = [u(m)/C]^2 + [u(V_i)/V_i]^2 + [u(V_e)/V_e]^2 + [u(rec)/rec]^2 + [u(V_s)/V_s]^2 \quad (5)$$

where each input of the concentration function [Eq. (4)] represents a source of uncertainty that contributes to combined uncertainty of the method and must be estimated.

The terms of this equation corresponding to volumetric uncertainty are easy to determine by measuring n times the same volume of each volumetric instrumental; their uncertainties can be estimated from Eq. (2). These uncertainties were estimated to be 1.84%, 1.67% and 2.15% for $u(V_s)/V_s$, $u(V_e)/V_e$ and $u(V_i)/V_i$, respectively.

The uncertainty of mass $u(m)$ is directly related to the uncertainty of the equation of regression $u(m)_1$ and to the preparation of the calibration mix used to plot the linear regression $u(m)_2$.

The mass of the pesticides (in the extract) injected into the GC is obtained directly by interpolation of the chromatographic area to this equation. The uncertainty of the equation of regression $u(m)_1$ [Eq. (6)] was calculated by applying the mathematical procedure [Eq. (1)] to regression equation, where the mass x is isolated:

$$u(m)_1 = u(x) = \{u_y 2(1/a)^2 + u_b 2(1/a)^2 + u_a 2[(y-b)/a^2]\}^{1/2} \quad (6)$$

where x is the mass injected into the GC (pg), y is a chromatographic area of a target pesticide and a and b are the slope and the intercept value, respectively, of the equation of regression. u_a and u_b are both calculated by using Eq. (2), where S is calculated directly from S_a and S_b (standard deviation of each term obtained directly from regression data analysis)

and n is the number of points used to calculate the equation of regression. On the other hand, u_y is obtained from repetitive injection experiments. At this level of concentration, the uncertainty of $u(m)_1$ is in the 0.001–0.002 ng/μl range for all compounds (1–2% as a relative uncertainty). The response of the MS detector itself is not repetitive along time and this is a new source of uncertainty which is necessary to estimate. In order to do it, a set of ten equations of regression of several pesticides (i.e. simazine and atrazine), calculated during 6 months, could establish a maximum variability of $u(m)_1$ along time of 3% for these pesticides, thus providing a good estimation for all other pesticides.

The uncertainty related to the preparation of calibration mix $u(m)_2$ was calculated by applying the mathematical procedure [Eq. (1)] to dilution equations and collecting as many laboratory instrumental data as possible. This relative uncertainty is 1.5% for all compounds as a maximum. Finally, the uncertainty of the mass $u(m)$ at 0.1 ng/μl level is calculated by the quadratic sum of the maximum uncertainty estimations of these two compounds [$u(m)_1$ and $u(m)_2$] and is in the 4–5% range for all compounds.

Finally, the uncertainty of recovery of each pesticide was calculated from validation data through repetitive experiments by using Eq. (2). This relative uncertainty is in the 3–7% range for all compounds but is higher for cyanazine (11%), β-endosulfan (9.1%) and trifluralin, dimethoate and terbutryn (8.3–8.6%).

The accumulation of expanded relative uncertainty U ($k=2$) in this analytical method is in the range 10–20% for most compounds. The greater contribution of uncertainty arises from the recovery and from the chromatographic quantification. Table 3 summarizes the uncertainty analysis for the pesticides studied.

The estimated uncertainty for all compounds proves that this method is suitable for multiresidue analysis of pesticides by using SPE-GC–MS in the working range and under the conditions established.

Table 3
Detailed estimation of uncertainty contributions in pesticide analysis for each compound

Pesticide	$u(V_s)/V_s$ (%)	$u(V_e)/V_e$ (%)	$u(V_i)/V_i$ (%)	$u(rec)/rec$ (%)	$u(m)/C$ (%)	u_c (%)	**U ($k=2$) (%)**
Dichlobenil	1.84	1.67	2.15	5.57	4.54	7.3	**14.6**
DIA	1.84	1.67	2.15	3.28	4.43	5.7	**11.4**
DEA	1.84	1.67	2.15	5.02	4.54	6.9	**13.8**
Trifluralin	1.84	1.67	2.15	8.35	4.39	9.6	**19.1**
Dimethoate	1.84	1.67	2.15	8.86	4.42	10.0	**20.0**
Simazine	1.84	1.67	2.15	7.22	4.40	8.6	**17.2**
Atrazine	1.84	1.67	2.15	3.91	4.38	6.1	**12.2**
Propazine	1.84	1.67	2.15	5.95	4.49	6.0	**12.0**
Lindane	1.84	1.67	2.15	3.73	4.44	5.4	**10.8**
Terbutylazine	1.84	1.67	2.15	2.69	4.45	7.6	**15.2**
Pirimicarb	1.84	1.67	2.15	2.80	4.60	5.5	**11.0**
Metribuzine	1.84	1.67	2.15	4.04	4.42	6.2	**12.4**
Alachlor	1.84	1.67	2.15	2.74	4.49	5.4	**10.9**
Terbutryn	1.84	1.67	2.15	8.58	4.39	9.8	**19.5**
Ethofumesate	1.84	1.67	2.15	5.18	4.46	7.0	**14.0**
Metolachlor	1.84	1.67	2.15	7.85	4.44	9.1	**18.3**
Chlorpyriphos-ethyl	1.84	1.67	2.15	5.09	4.46	6.9	**13.8**
Cyanazine	1.84	1.67	2.15	11.00	4.37	11.9	**23.9**
Metazachlor	1.84	1.67	2.15	7.52	4.43	8.9	**17.7**
Pendimethalin	1.84	1.67	2.15	4.44	4.43	6.5	**12.9**
α-endosulfan	1.84	1.67	2.15	7.78	4.49	9.1	**18.2**
β-endosulfan	1.84	1.67	2.15	9.13	4.47	10.3	**20.5**

$u(V_s)/V_s$, $u(V_e)/V_e$ and $u(V_i)/V_i$ are the relative uncertainty of the volume of sample, extract and injection, respectively. $u(m)/C$ is the relative uncertainty of the mass. u_c and U are the estimated combined relative standard uncertainty and the expanded uncertainty ($k=2$) of the method for each compound studied, respectively.

3.3. Results

The occurrence of pesticides in raw water, treated water and groundwater during the year 2000 in Barcelona and surrounding areas is summarized in Table 4. They include samples from the Llobregat, Ter and Besòs rivers. Fig. 1 shows TIC and SIR GC–MS chromatograms of a real sample.

3.3.1. Samples from the Llobregat river (raw and treated water)

Simazine, atrazine and its metabolite DIA are relatively frequent in the raw water entering the water treatment plants WTP1 and WTP2. The levels found for these pesticides are always below 0.1 μg/l. These compounds present higher values during the period February–June, which coincides with their period of field application, whereas declining values are observed for the rest of the year. Two other compounds — dimethoate and terbutryn — were seldom detected in the river water entering WTP1. Both pesticides came probably from the Anoia river, a small tributary of the Llobregat river. These compounds were previously identified after fish mortality in this tributary but the origin of dumping is still unknown. As grab samples were analyzed, there was not necessarily correlation between raw and treated water and no conclusion about the treatment process can be inferred from data depicted in Table 4. Simazine and DIA were the two main compounds identified in treated water from WTP1 and WTP2 and were detected few times in raw water.

Table 4
Occurrence of pesticides in raw water, treated water and groundwater (in μg/l) in the Barcelona area (NE Spain) during the year 2000. Water samples came from WTP1, WTP2, WTP3 and groundwater

	WTP1 raw water		WTP2 raw water		Groundwater from the Llobregat aquifer		Groundwater from the Besòs aquifer	
Compound	min.–max.	Freq.	min.–max.	Freq.	min.–max.	Freq.	min.–max.	Freq.
Simazine	<0.025–0.084	6	<0.025–0.043	1	<0.025–0.073	28	0.046–0.164	15
Atrazine	<0.025–0.029	1	–	n.d.	<0.025–0.025	3	<0.025–0.059	9
Terbutylazine	–	n.d.	–	n.d.	<0.030–0.053	2	<0.030–0.083	8
DIA	<0.025–0.062	6	–	n.d.	<0.025–0.025	1	<0.025–0.063	8
DEA	–	n.d.	–	n.d.	–	n.d.	<0.025–0.036	2
Terbutryn	<0.040–0.070	3	<0.040–0.110	1	–	n.d.	–	n.d.
Metolachlor	–	n.d.	–	n.d.	–	n.d.	–	n.d.
Dimethoate	<0.060–0.154	2	–	n.d.	–	n.d.	–	n.d.
Samples analyzed		20		12		43		15

	WTP1 treated water		WTP2 treated water		WTP3 treated water	
Compound	min.–max.	Freq.	min.–max.	Freq.	min.–max.	Freq.
Simazine	<0.025–0.043	3	<0.025–0.034	1	–	n.d.
Atrazine	–	n.d.	<0.025–0.025	1	0.031–0.062	18
Terbutylazine	–	n.d.	–	n.d.	–	n.d.
DIA	<0.025–0.025	1	<0.025–0.073	3	–	n.d.
DEA	–	n.d.	–	n.d.	<0.025–0.033	6
Terbutryn	–	n.d.	–	n.d.	–	n.d.
Metolachlor	–	n.d.	–	n.d.	<0.035–0.040	5
Dimethoate	–	n.d.	–	n.d.	–	n.d.
Samples analyzed		23		10		18

min.–max.: minimum and maximum concentration; freq.: number of positive samples; n.d.: not detected.

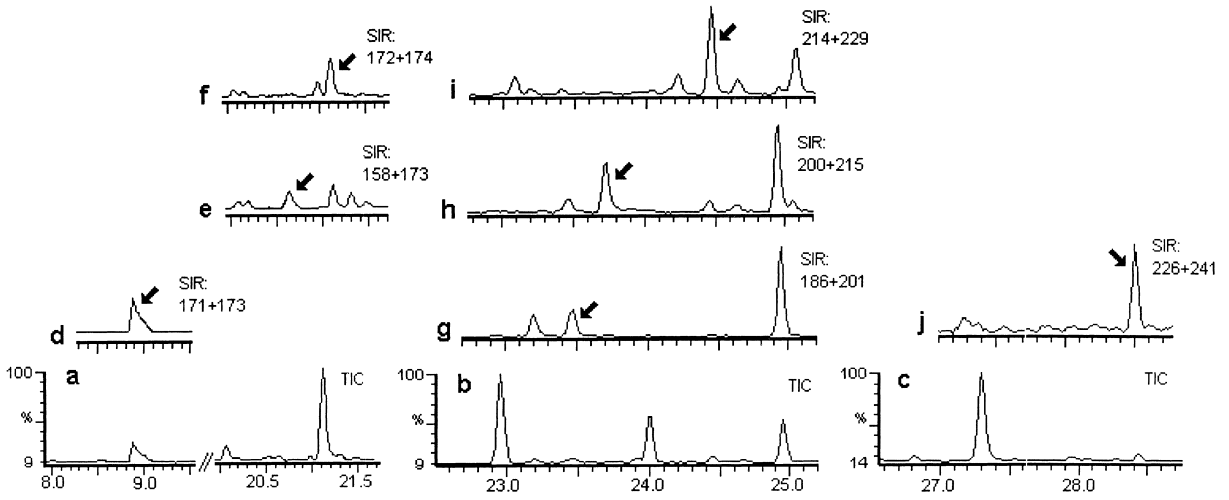

Fig. 1. (a–c) Total ion GC–MS chromatogram of a groundwater extract from Besòs water. (d–j) Reconstructed ion chromatograms of dichlobenil (*d*), DIA (*e*), DEA (*f*), simazine (*g*), atrazine (*h*), terbutylazine (*i*) and terbutryn (*j*).

3.3.2. Samples from the Ter river (treated water)

The samples analyzed are WTP3 treated water collected in the east part of the city and surrounding areas without blending with treated water from the other WTPs. Atrazine, its metabolite DEA and metolachlor are the main compounds identified at levels lower than 0.1 μg/l. The agricultural activity together with different tannery and leather industries, which dump their wastewaters to the tributaries of the Ter river, are probably the major contributors to the presence of pesticides in the dam systems of the Ter river which serves raw water through a pipeline to WTP3.

3.3.3. Groundwater samples

Samples from the aquifer of the Llobregat and Besòs rivers were analyzed. As expected, simazine was the main triazine identified with terbutylazine, atrazine and its metabolites DEA and DIA, in groundwater from the Llobregat river aquifer. This groundwater is incorporated into the treatment process of the WTP1 during drought periods before the carbon filtration step. It is feasible that some of the simazine detected in treated water from WTP1 came from the inefficient removal of this compound during the filtration process when groundwater is mixed with pretreated river water. On the other hand, groundwater from the Besòs river aquifer reflects the intensive agricultural activity in this area. Simazine was always detected with a peak level of 0.164 μg/l. Terbutylazine, atrazine as well as its main metabolites were detected at more than 50% of the samples analyzed. Table 5 shows physicochemical parameters of all these raw and treated waters.

4. Conclusions

A multiresidue method developed to determine the presence of 22 largely used pesticides in Europe (SMT-CT96-2142) has been used to analyze their presence in NE Spanish drinking and related waters. In addition, the measurement of the uncertainty for their determination has been carried out for all pesticides by using the ‘top-bottom’ method, which estimates the accumulation of uncertainty of several stages of the process. The expanded relative uncertainty for the method is in the range of 10–20% for most compounds being the main contribution of uncertainty the recovery and the chromatographic quantification. The study shows that simazine, atrazine and, to a lesser extent, DIA are the main compounds found in raw, treated and groundwater at concentration levels lower than 0.1 μg/l.

Table 5
Physicochemical parameters of raw and treated waters

	pH	Conductivity μS/cm	Cl^- mg Cl^-/l	NH_3 mg NH_3/l	TOC mg C/l
WTP1 raw water	8.19	1530	390	0.91	6.8
WTP2 raw water	8.14	1405	299	0.83	4.9
Groundwater from the Llobregat aquifer	7.26	1865	380	0.03	2.01
Groundwater from the Besòs aquifer	7.48	1882	329	4.01	n.d.
WTP1 treated water	7.41	1616	369	<0.1	3.02
WTP2 treated water	7.42	1437	308	<0.1	2.07
WTP3 treated water	7.47	510	70	<0.1	2.01

TOC: Total organic carbon; n.d.: Not determined.

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