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Analysis of thiabendazole, 4-*tert*-octylphenol and chlorpyrifos in waste and sewage water by direct injection – micellar liquid chromatography

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A micellar liquid chromatographic method has been developed for the simultaneous quantification of the pesticides thiabendazole and chlorpyrifos, as well as an alkylphenol, which is included in pesticide formulations, *i.e.*, 4-*tert*-octylphenol, in water. A sample was filtered and directly injected, avoiding large extraction steps using toxic solvents, thus expediting the experimental procedure. The contaminants were eluted without interferences in <17 min, using a mobile phase of 0.15 M sodium dodecyl sulfate – 6% 1-pentanol buffered at pH 3, running through a C18 column at 1 mL min^{-1} under the isocratic mode. This optimal mobile phase was selected using a statistical approach, which considers the retention factor, efficiency and peak shape of the analytes measured in only a few mobile phases. The detection was carried out by measuring absorbance at 220 nm. The method was successfully validated in terms of specificity, calibration range (0.5–10 mg L^{-1}), linearity (r^2 > 0.994), limit of detection and quantification (0.2–0.3; and 0.5–0.8 mg L^{-1}, respectively), intra- and interday accuracy (95.2–102.9%), precision (<8.3%), and ruggedness (<9.3%). The stability in storage conditions (at least 14 days) was studied. The method was safe, inexpensive, produced little pollutant and has a short analysis time, thus it is useful for the routine analysis of samples. Finally, the method was applied to analyse wastewater from the fruit-processing industry, wastewater treatment plants, and in sewage water belonging to the Castelló area (Spain). The results were similar to those obtained by an already reliable method.

1. Introduction

Pesticide formulations are used in agriculture and food-processing plants to protect crops during growing, storage, and in gardening to maintain house plants, from annoying pests. They are made of a pesticide, as the active principle component, mixed with other materials such as stabilizers, solvents, adjuvants, foaming agents, dispersants, suspensors or emulsifiers.[1,2] Non-ionic alkylphenol polyethoxylates (APEs) are among the surfactants that are most commonly included in pesticide formulations. They are also added in household detergents,[3] cosmetics and office products.[4] Because of their proven toxicity, persistency in the environment and bioaccumulation, pesticides[5,6] and APEs[3,4] represent an important source of contamination of natural water.

These hazardous compounds are incorporated into agricultural and food-processing plant waste and municipal sewage water, which are further processed by wastewater treatment plants (WWPT) to remove the contaminants. Depending on the pollutant, its concentration in the influent water and the purification technique applied in the WWPT, the elimination may be incomplete. Hence, some amount of pesticides and APEs can remain in the effluent water, which is discharged to the river.[7,8] The occurrence of these contaminants in natural water causes serious damage to local flora and fauna.[2,3] The population is also directly exposed to this contamination by accidental inhalation, dermal and oral contact with polluted water,[3,9] and through the food chain, by the consumption of edible tissue of animals and plants grown with contaminated water.[10,11] Actually, these chemicals are cataloged as "Emerging Pollutants", hazardous compounds that have to be controlled and regulated due their potential environmental and health hazards. The European Union, through the "EU Water Framework Directive"[12] and the US Environmental Protection Agency[13] have implemented programs and policies to monitor these compounds in surface water.

Thiabendazole (TBZ) is a fungicide and antiparasitic, which is largely used as a post-harvest preservative for various fruits and vegetables. Thiabendazole health effects include damage

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to red blood cells, liver and thyroid. It is even carcinogenic at high concentrations.[14] Chlorpyrifos (CPF) is an insecticide, which is extensively used pre-harvest in agriculture to protect crops such as cotton, corn, almonds, orange and apples, and in households to protect ornamental plants, lawn, pets and wooden objects.[5] CPF is quite toxic and it causes diseases and disrupting effects on the nervous system with short term contact.[15] The short APE 4-*tert*-octylphenol (4-*t*OP) is a product of degradation by aerobic hydrolysis of long APEs spiked in formulations.[16] APEs show endocrine disruption effects, thus altering the hormonal system. In addition, 4-*t*OP shows higher toxicity and bioaccumulation than its long APE precursors.[3,4] These compounds are largely used in the Castelló area, due to its strong fruit agriculture and fruit-processing industry, which introduces a high risk of water contamination. Thus, the monitoring of TBZ, CPF and 4-*t*OP in waste and sewage water is required to protect population health and the environment.

A high amount of analytical methodologies has been developed to detect pesticides[17] and alkylphenols[18,19] in several types of water. Among them, those based on both gas chromatography (GC) and liquid chromatography (LC) are predominant. HPLC coupled to mass spectrometry (MS) has been proposed for routine analysis of pesticides[20] and APEs[21–24] in water samples, although GC-MS is still being used.[25,26] However, a mass spectrometer is an expensive instrument, hence, the analyses of water samples are high-priced. HPLC coupled with UV-visible absorbance (DAD) is an economic alternative and has been shown to be successful in several reports.[27–30] Waste and sewage water usually contain suspended sludge and oily compounds, and requires sample preparation to avoid the introduction of harmful substances in the chromatographic system. The experimental procedure involves tedious and time consuming clean-up steps, such as solid/liquid[20–22,24,28,30] and liquid/liquid[27,29] extraction, which increases toxic waste and the risks related to the handling of hazardous reagents. The introduction of additional steps can also cause sample loss or experimental error. Recently, new efforts have been taken to develop analytical methodologies to avoid these problems.[31]

Micellar liquid chromatography, using hybrid mobile phases containing sodium dodecyl sulfate (SDS) as the surfactant and a short-chain alcohol, has been demonstrated as an interesting alternative to hydroorganic-RP-HPLC.[32] Micellar solutions are able to solubilize compounds within a wide range of polarities. Therefore, samples with hydrophobic compounds can be directly injected, without the risk of column damage. Moreover, the surfactant monomer coat on the external layer of the stationary phase, changes its characteristics. The analyte is partitioned between three environments (stationary phase, mobile phase and micelles), thus improving the versatility of MLC.[33] The strong reproducibility and stability of the chromatographic behavior of the analytes allows the prediction of the solute retention using a statistical model, from the experimental data, which can be obtained in several mobile phases, thus expediting the optimization of the mobile phase composition. Moreover, micellar mobile phases are non-flammable, less toxic, more environmentally friendly, and relatively inexpensive than those used in hydroorganic-HPLC.[34] Micellar liquid chromatography has been previously proposed to detect chemical pollutants in wastewater,[35] and the pesticide carbaryl[36] in environmental water.

The aim of this work is to develop a rapid, easy-to-handle, inexpensive, environmentally friendly and reliable method to detect the pesticides TBZ, CPF and the short APE 4-*t*OP in water samples, in order to apply it to routine analyses. The features of MLC are exploited to allow the direct injection of the sample and resolve the mixture of analytes in a short chromatographic run. The method is validated in terms of calibration, linearity, sensitivity, intra- and interday accuracy and precision, ruggedness and stability to prove its reliability.[37] Finally, the developed analytical method was used to quantify the analytes in WWPT influent and effluent, industrial waste from the fruit-processing industry, and sewage water samples, which were collected at several points in the Castelló area. The results are compared with those obtained by a reference method based on LC-MS.

2. Material and methods

2.1 Chemicals and equipment

Standards of TBZ, CPF and 4-*t*OP (purity > 99.0%), were purchased from Dr Ehrenstorfer-Schäfers (Augsburg, Germany). The structures and main physicochemical characteristics of these compounds are shown in Table 1. The characteristics of these compounds are also included in Table 1. SDS (purity > 99.0%), methanol, 1-butanol, 1-pentanol (HPLC grade) were obtained from Scharlab (Barcelona, Spain). Hydrochloric acid, sodium hydroxide and 1-propanol were supplied by Panreac (Barcelona, Spain). The additives triethylamine (TEA) and 1-ethyl-3-methylimidazolium tetrafluoroborate ($EMIMBF_4$), both of HPLC grade, were obtained from J.T. Baker (Deventer, The Netherlands) and Sigma-Aldrich (St. Louis MO, USA), respectively. Ultrapure water was in-laboratory produced from deionized water using an ultrapure water generator device, Millipore S.A.S. (Molsheim, France). This ultrapure water was used in all aqueous solutions.

2.2 Preparation of solutions and mobile phases

The stock solutions of the pesticides were prepared by weighing a portion of the pesticide and dissolving it in methanol, in order to obtain concentrations of 100 μg mL^{-1}. Working solutions were prepared by diluting these stock solutions in methanol to reach the desired concentration. All the solutions were protected from light and stored at 4 °C.

The micellar mobile phases were prepared by dissolving the appropriate amount of SDS and sodium dihydrogenphosphate in ultrapure water. An adequate volume of TEA or $EMIMBF_4$ was added, and then the pH was adjusted by adding drops of HCl or NaOH solution to reach the desired value. Furthermore, an adequate volume of short-chain alcohol was added, the solution was filled up to the final volume with ultrapure water, ultrasonicated and filtered.

Table 1 Structure and physicochemical parameters of the analytes

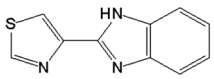
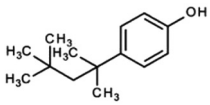
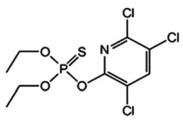
Compound	Structure	pK_a	Charge at pH = 3	log $P_{o/w}$
Thiabendazole[15]		4.73/12.00	+1	1.62
4-*tert*-Octylphenol[3]		10.7	0	4.12
Chlorpyrifos[17]		Not applicable	0	4.70

Table 2 Calibration and sensitivity parameters of the studied pollutants[a]

Compound	Slope	Intercept	r	r^2	LOD	LOQ
Thiabendazole	0.8 ± 0.1	−0.05 ± 0.06	0.997	0.9946	0.20	0.5
4-*tert*-Octylphenol	0.60 ± 0.03	−0.04 ± 0.03	0.998	0.9966	0.25	0.6
Chlorpyrifos	0.42 ± 0.02	−0.03 ± 0.06	0.9993	0.9993	0.30	0.8

[a] Slope and y-intercept: average value ± standard deviation, concentrations in μg mL^{-1}; n = 5.

All the solutions and mobile phases were filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA, USA).

2.3 Apparatus and instrumentation

The solid standard and reagents were weighted on a Mettler-Toledo analytical balance (Greifensee, Switzerland). A GLP 22 potentiometer (Crison, Barcelona) equipped with a combined Ag/AgCl/glass electrode was used to measure pH values. The ultrasonication of the mobile phases was performed in an ultrasonic bath; model Ultrasons-H (Selecta, Abrera, Spain).

The separation and quantification was performed using an Agilent Technologies HP 1100 Series (Palo Alto, CA, USA) chromatographic system equipped with an isocratic pump, a degasser, an auto sampler and a UV-visible variable wavelength detector (VWD). The signal was obtained by a personal computer connected to the chromatographic system using an Agilent Chemstation version B.01.01. The chromatographic parameters such as retention time (t_R, min), peak area (A, arbitrary units), dead time (t_0, min), retention factor (k), efficiency (N, theoretical plates) and asymmetry (B/A) were obtained from the registered chromatograms using the Michrom software.[38] The meaning of these chromatographic parameters can be found in ref. 39.

2.4 Chromatographic conditions

The stationary phase was coated on a Kromasil C18 column (125 × 4.6 mm, 5 μm, 100 Å) from Scharlab. The mobile phase was an aqueous solution of 0.15 M SDS – 6% 1-pentanol buffered with 0.01 NaH_2PO_4 at pH 3, running under the isocratic mode at 1 mL min^{-1} at room temperature. The injection volume was 20 μL and the absorbance detection wavelength was set at 220 nm. The special care required for the chromatographic system when dealing with micellar mobile phases can be seen in ref. 40 Under these conditions, the column has a lifespan of nearly 1000 injections.[40]

2.5 Sample treatment

Water samples were provided by FACSA, the company which manages the water monitoring and treatment in the Castelló province in Spain. The samples were collected during the February–May period from several places where the presence of TBZ, CPF or 4-*t*OP is suspected: influent and effluent of WWPT, fruit-processing plant wastewater and sewage water (Table 2). The samples were placed in a fridge protected from light (amber glass) until analysis.

Prior to analysis, sample water or standard solutions were taken out of the fridge and maintained for 30 min to reach room temperature. Then, they were filtered and directly injected into the chromatographic system.

3. Results and discussion

3.1 Optimization of the chromatographic conditions

The column, injection volume and flow rate were taken as the usual conditions in MLC, whereas the composition of the mobile phase and the detection condition were optimized. A standard solution containing 2 μg mL^{-1} of TBZ, CPF and 4-*t*OP was used for optimization.

3.1.1 Optimization of the pH. The pH was selected in the working range of the column (1.5–7.5). The mobile phase was buffered to avoid variation of pH when the sample was injected in the mobile phase flow.

Three mobile phases as described in section 2.4, were buffered to pH 3, 5 and 7 and tested. At the three pH, the retention times were similar for the three studied compounds. However, a strong tailing was observed for TBZ at pH 5 and 7, whereas the peak shape was quite Gaussian at pH 3. For CPF and 4-*t*OP, the peak shape was comparable at the three pH. As a consequence, pH 3 was selected for the analyses.

3.1.2 Selection of the organic modifier. According to the strong hydrophobicity of 4-*t*OP and CPF, a pure SDS solution would be unable to elute them from a C18 column in a reasonable retention time.[41] Therefore, SDS/1-propanol SDS/1-butanol and SDS/1-pentanol hybrid mobile phases were tested.

The mobile phases containing the maximal concentration recommended for SDS and each short-chain alcohol were tested: 0.15 M SDS/12.5% 1-propanol, 0.15 M SDS/7% 1-butanol and 0.15 M SDS/6% 1-pentanol.[32] In the three mobile phases, the elution order was: t_R(TBZ) < t_R(4-*t*OP) < t_R(CPF), and these retention times increases when the MW of the alcohol decreases. Finally, the mobile phases containing 1-butanol and 1-propanol were discarded because the analysis time was too high. Thus, 1-pentanol was selected.

3.1.3 Optimization of SDS/1-pentanol concentration. The concentrations of SDS and 1-pentanol were simultaneously optimized using an interpretative strategy. The experimental design consists of four mobile phases containing a combination of the minimum and maximum amount recommended for SDS and 1-pentanol in MLC, and the average value. Therefore, the mobile phases tested were SDS (M)/1-pentanol% (v/v): 0.05/2; 0.05/6; 0.1/4; 0.15/5 and 0.15/6.[32] The experimental chromatographic parameters: (retention factor; efficiency and asymmetry) for each mobile phase were taken for the three analytes. From these preliminary studies, it was deduced that TBZ, 4-*t*OP and CPF show a bending behavior face to SDS, and the retention factor and the efficiency decrease at higher SDS concentrations. As expected, the elution power and the peak shape increased with larger amount of 1-pentanol.

The more adequate mobile phase composition was obtained using a statistical model. The relationship between the retention factor of a specific compound and the SDS ([M]) and 1-pentanol (ϕ) concentrations of the mobile phase are related by the following equation:[32]

$$k = \frac{K_{AS}\dfrac{1}{1+K_{AD}\phi}}{1+K_{AM}\dfrac{1+K_{MD}\phi}{1+K_{AD}\phi}[M]}$$

The constants signify partition coefficients between phases. Where: K_{AS}, is the partition constant between the stationary phase and aqueous environment; K_{AM}, is the partition coefficient between the micelle and the aqueous environment, and K_{AD} and K_{MD}, are the relative variation in the solute concentration in pure water and micelles due to the presence of 1-pentanol, as compared to a pure micellar solution.[42] Another equation allows for the modeling of the peak shape (N and B/A) at several SDS/1-pentanol concentrations.[32]

For each analyte, the experimental values of k, N and B/A, which were obtained from the five tested mobile phases were processed by the Michrom software as "calibration levels" in order to calculate the constants of the equations. Therefore, the mathematical model was able to predict the chromatographic behavior (the values of k, N and B/A) of TBZ, 4-*t*OP and CPF in mobile phases containing intermediate SDS and 1-pentanol concentrations, 0.05–0.15 M, and 2–6%, respectively. The software also predicted the resolution of each pair (r_{ij}), which was calculated using the valley peach criterion, and the global resolution (R) was taken as the least r_{ij}. This information was used to draw simulated chromatograms, in order to allow the operator to visualize the variations of k, N and B/A of the analytes when the SDS and 1-pentanol concentrations in the mobile phase change.[32,38]

According to the statistical model, using a mobile phase of 0.15 M SDS – 6% 1-pentanol at pH 3, the three analytes would be completely resolved (R = 1) in the minimum analysis time (<20 min). A solution containing 2 μg mL^{-1} of each studied pollutant was analyzed. The experimental chromatographic parameters (t_R; N and B/A) were as follows: TBZ (3.82 min; 1490; 0.97), 4-*t*OP (7.43 min; 1340; 1.08) and CPF (14.16 min; 1110 and 1.06). The obtained chromatogram can be seen in Fig. 1A. As predicted, the mixture was completely resolved in an adequate time (<17 min), thus proving the high specificity of the method. The errors in the expected retention factors were below 6%.

3.1.4 Optimization of additive concentration. The additives triethylamine (a tertiary amine) and 1-ethyl-3-methylimidazolium tetrafluoroborate (an ionic liquid) has been used in liquid chromatography to block the protonated silanol groups.[43] This avoids their interaction with the column, thus preventing the formation of tailing and improving peak shape. Therefore, two mobile phases containing 0.5% of TEA and $EMITBF_4$ were tested. In both cases, the retention factors of the analytes increased without improvement in the peak shape when compared with the mobile phase selected in section 3.1.3. For this reason, the use of these additives was discarded.

3.1.5 Optimization of the detection conditions. The mixture of TBZ, 4-*t*OP and CPF was analyzed through the previously selected optimized conditions, at wavelengths ranging from 200 and 300 nm by measuring at 10 nm intervals. Thus, we obtained the absorbance of each compound in the chemical environment formed by the already selected micellar mobile phase. A strong absorbance was observed by the three analytes at 220 nm, with low baseline noise. Therefore, this value was taken for the analysis, and the whole chromatogram was registered at the same wavelength.

3.1.6 General discussion. One of the main features of the optimized procedure is the reduction of time for analysis and the simplification of the experimental procedure. This is possible because of the possibility to directly inject the sample, which allows the elimination of intermediate extraction steps. In addition, the elution of the analytes was performed in <17 min using the isocratic mode, due to the use of micellar mobile phases. Thus, the stabilization time between two

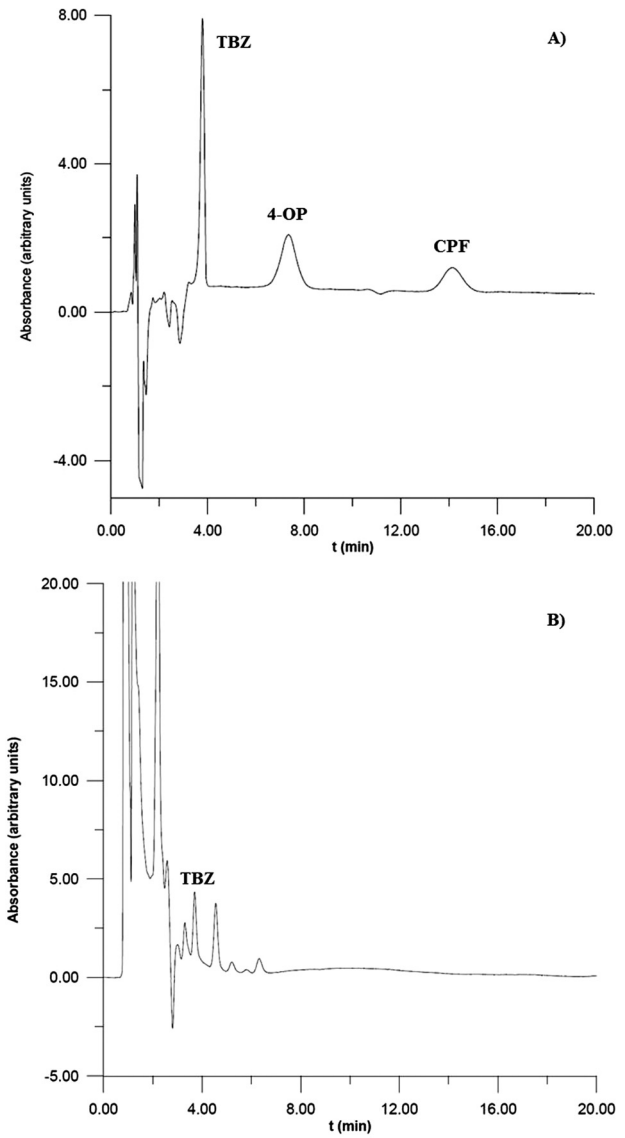


Fig. 1 Chromatogram obtained by the analysis of: (A) a mixture of 2 mg L^{-1} of TBZ, 4-*t*OP and CPF and (B) water sample 13 collected from the wastewater collector basin in the fruit-processing plant Invicto, Villarreal, Spain. Chromatographic conditions: C18 column, mobile phase 0.15 M SDS – 6% 1-pentanol – pH 3; detection at 220 nm.

successive injections, which is required in the gradient mode, is not needed. This characteristic facilitates the successive analysis of a large amount of samples.

Another interesting advantage is the minor environmental impact of the analysis and the reduction of the risks related to handling hazardous reagents. The experimental procedure does not require any chemicals, and the optimized mobile phase uses a lesser amount of organic solvents (6% 1-pentanol), than typically used in hydroorganic HPLC (up to 100%).

The analysis can be performed at a low cost because the method only requires basic chromatographic instrumentation and a small amount of inexpensive reagents are used. In addition, the analysis of a large amount of samples per day is possible.

All of these features make the method feasible even for laboratories with low economic power, thus allowing them to sell these analyses at low prices, and also extremely useful for the routine analysis of water samples for pollution monitoring.

3.2 Method validation

The method was validated to check the quality of the quantitative data and evaluate its performance. The validation parameters were: calibration range, linearity, intra- and interday accuracy and precision, ruggedness and stability.[37] The entire calibration was performed in ultrapure water.

3.2.1 Calibration and sensitivity. For calibration purposes, eight solutions containing increasing concentrations of TBZ, 4-*t*OP and CPF in the range 0.5–10 μg mL^{-1} were analyzed in triplicate. The slope, *y*-intercept, regression coefficients and determination coefficients were obtained by plotting the peak area (average of three measurements) *vs.* concentration using the least-square linear regression method. The study was repeated five days over a 3-month period, by preparing standard solutions each time. The calibration curves were taken as the average values of these five regression curves and the results are shown in Table 2. Excellent linearity ($r > 0.997$ and $r^2 > 0.994$) was observed for the three contaminants in the range LOQ – 10 μg mL^{-1} (see below).

The limit of detection (LOD), is the lowest pesticide concentration in a sample, which produces a response that is detectable above the noise level of the system. The LOD was obtained by visual appreciation following the 3 signal-to-noise ratio criterion, which is the concentration value providing a signal 3 times the baseline noise. The baseline noise was measured for each analyte, by analyzing a blank and measuring the width of the baseline at the corresponding retention time.[37] The LOQ was taken as the lowest point of the calibration curve with a precision <20% and accuracy between 80% to 120% (see section 3.2.2).[37] The results can be seen in Table 2. The values indicate that the method is able to detect the presence of these compounds in contaminated waste and sewage water.

3.2.2 Accuracy and precision. The intra- and inter-day accuracy and precision were determined at three concentration levels (1; 2 and 5 μg mL^{-1}). The intra-day accuracy was calculated as the ratio concentration provided by the method (average value of 6 analyses taken the same day)/true value. The intraday precision was the RSD of the peak area obtained by six analyses on the same day. Interday accuracy was calculated as the average of the intraday values obtained in five different days over a 3-month period, using renewed solutions. Interday precision was measured as the RSD of the peak area of days over a 3-month period. The results are shown in Table 3. The method shows high recovery (95.2%–102.9%) and low variability (<8.3%) in the determination of TBZ, 4-*t*OP and CPF in water, thus confirming the reliability of the quantitative data.

3.2.3 Ruggedness. The ruggedness was examined by considering the variation in the elution power and the sensitivity area face to minor, but deliberate variations in the surfactant

Table 3 Intra- and inter-day accuracy and precision for TBZ, 4-OP and CPF

Compound	Concentration (μg mL^{-1})	Intra-day[a]		Inter-day[b]	
		Accuracy (%)	Precision (RSD, %)	Accuracy (%)	Precision (RSD, %)
Thiabendazole	1	103.3	0.7	101.8	1.4
	2	95.5	0.8	99.4	1.4
	5	101.2	0.4	96.9	3.4
	1	110.1	4.4	102.1	7.5
4-*tert*-Octylphenol	2	93.8	1.5	96.7	1.1
	5	98.2	0.3	97.5	1.5
	1	102.9	3	100.5	8.3
Chlorpyrifos	2	88.2	3.6	95.2	3.3
	5	102.8	0.5	101.3	1.5

[a] $n = 6$. [b] $n = 5$.

concentration, 1-pentanol amount, pH and flow rate. To study the influence of a determinate condition, a standard solution containing 2 μg mL^{-1} of each analyte was analyzed in three mobile phases: at its optimal value, slightly under and slightly over, while maintaining the other conditions constant. Thus, the influence of each parameter was separately studied. The considered ranges were as follows: SDS concentration (0.145–0.155 M), 1-pentanol (5.9%–6.1%), pH (2.9–3.1) and flow rate (0.95–1.05 mL min^{-1}) in triplicate and the RSD of the measured retention times and peak areas were then calculated.

The small experimental oscillations in the main chromatographic conditions that may happen during routine analysis had no significant influence in the retention time (RSD < 5.1%) and the peak area (RSD < 9.3%) of TBZ, 4-*t*OP and CPF.

3.2.4 Stability. The stability of the analytes in water was studied at +60 °C and in fridge storage conditions (+4 °C in darkness). Although 60 °C is rarely reached in real situations, the results would provide interesting information about the thermostability of the analytes. In both cases, a solution containing 1 μg mL^{-1} of TBZ, 4-*t*OP and CPF was used.

The pollutant standard solution was heated to 60 °C in a water bath. An aliquot was analyzed at 20 min intervals during a 3 h period. The peak area corresponding to the contaminants remained almost constant. Therefore, TBZ, 4-*t*OP and CPF are quite thermostable and cannot be removed by heating.

The standard solution was kept in a fridge, at +4 °C and in darkness. Daily, an aliquot was analyzed, and no significant diminution in the peak area was observed up to 14 days. Therefore, a water sample can be collected and stored in a fridge until 14 days prior to analysis, without analyte degradation.

3.3 Analysis of real samples from sewerage and wastewater. The developed method was applied to the analysis of samples provided to us by FACSA. The samples were collected from sewerage, industrial waste, and influent and effluent WWPT water from several towns located in the Castelló area (Spain), where the occurrence of TBZ, 4-*t*OP and CPF is suspected. We analyzed the water samples at a maximum of three days after we received them. Previously, FACSA analyzed the samples using its own standardized LC-MS method. For confidentiality reasons, FACSA has not provided us the characteristics of this method. The origin of each sample and the content of TBZ, 4-*t*OP and CPF can be seen in Table 4. Despite the presence of suspended sludge in several samples, neither obstruction nor damage was noticed in the column, needle or tubes. Fig. 1B shows the chromatogram obtained by analyzing sample 13, which indicates the other water contaminants were eluted far from the retention time of the analytes.

The concordance of the results obtained by the two methods was evaluated by plotting the data obtained by MLC *vs.* those obtained by LC-MS, using least-square linear regression.[44] Only the samples providing reliable concentration values (over LOQ) were taken. The obtained curve was:

$$[\text{MLC}] = (1.13 \pm 0.08)\,[\text{LC-MS}] + (-0.09 \pm 0.08)$$
$$r^2 = 0.96 \text{ freedom degrees} = 9$$

The two values show an adequate correlation. A statistical hypothesis test was performed to assess the equivalence of the two values of each pair: null hypothesis H_0 slope = 1 and *y*-intercept = 0. Considering a significance level of $\alpha = 0.05$ and a two-tailed test, the tabulated students-*t* test value was 2.26 ($t_{0.05;9;\ 2\text{tails}}$). Thus, the confidence intervals were [0.96 to 1.34] and [−0.28 to 0.13] for the slope and *y*-intercept, respectively, thus the null hypothesis was accepted. Consequently, the results obtained by our MLC method were close to those obtained by FACSA from LC-MS. Although the sensitivity is lower, the analysis can be performed at a lower price. Moreover, the MLC methods can be applied to samples with a high contamination degree.

CPF was only detected in one sample, indicating that it remains in crops and sludge, rather than reaching water. We can see that TBZ occurs in almost all samples, due to its extended use. In fact, even the sewerage which does not receive agricultural waters contained TBZ. The contamination of the sewerage water which received wastewater from fruit production was slightly higher, indicating that pesticides are moderately applied to crops and they arrive diluted to the sewerage.

The wastewater from the fruit-processing plants showed a moderate/low concentration of TBZ and 4-*t*OP, indicating that these industries partially purge the wastewater before

Table 4 Concentrations (μg mL^{-1}) of TBZ, 4-*t*OP and CPF detected in real water samples[a]

Origin of water sample	Sample	Location	TBZ		4-*t*OP		CPF	
			MLC	LC-MS	MLC	LC-MS	MLC	LC-MS
Sewage receiving agricultural wastewater	1	Vila-real	<0.5	0.29	0.65	0.75	n.d.	0.24
	2	La Vilavella	<0.5	0.41	n.d.	n.d.	n.d.	n.d.
	3	Betxí I	<0.5	0.12	n.d.	0.1	n.d.	0.14
	4	Betxí II	<0.5	0.23	n.d.	0.21	n.d.	n.d.
	5	Onda	<0.5	0.30	n.d.	0.15	n.d.	n.d.
Sewage not receiving agricultural wastewater	6	Alcora	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	7	Nules	<0.5	0.04	n.d.	n.d.	n.d.	n.d.
	8	Vila-real I	<0.5	n.d.	n.d.	0.21	n.d.	n.d.
	9	Alcora	<0.5	0.08	n.d.	0.14	n.d.	n.d.
	10	Vila-real II	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	11	Onda	n.d.	n.d.	n.d.	0.14	n.d.	n.d.
Collector basin of wastewater from a fruit processing plant	12	Real Export (Vila-real)	1.1	0.93	n.d.	n.d.	< 0.8	0.18
	13	Invicto (Vila-real)	0.9	0.85	n.d.	0.15	n.d.	0.12
	14	Serifruit (Vila-real)	0.5	0.42	0.6	0.63	n.d	n.d.
	15	Eurococi (Betxí)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Wastewater from WWPT	16	Influent (Nules-La Vilavella)	<0.5	0.52	2.0	1.8	n.d.	0.21
	17	Effluent (Nules-La Vilavella)	<0.5	0.12	n.d.	n.d.	n.d.	n.d.
	18	Influent (Vora Riu)	1.9	1.71	0,8	0.71	n.d.	0.12
	19	Effluent (Vora Riu)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	20	Influent (Mancomunada OBVA)	0,8	0.84	0.8	0.88	n.d.	n.d.
	21	Decanted influent (Mancomunada OBVA)	< 0.50	0.41	1.1	1.2	n.d.	n.d.
	22	Effluent (Mancomunada OBVA)	n.d.	0.12	n.d.	n.d.	n.d.	n.d.

[a] n.d. = not detected (<LOD).

discharge. The influent samples from WWPT show higher concentrations than the effluent, thus confirming that the analytes are removed from wastewater and ensuring the validity of the water purification treatment.

4. Conclusions

The obtained data indicate that micellar liquid chromatography can be used to analyze TBZ, 4-*t*OP and CPF in highly contaminated waste and sewerage waters. The use of an interpretative strategy base on chemometrics has allowed the optimization of the two main parameters (SDS and 1-pentanol), by testing only five mobile phases. The main features of the developed method are the direct injection of the sample, after filtration, and the quick elution of the studied pollutants without overlapping in less than 17 min. The method was validated in terms of specificity, calibration range, linearity, accuracy, precision and ruggedness, and was successfully compared with an LC-MS established method, thus confirming its reliability. Besides, the method is safer for the operator and is environmental friendly, thus making it more attractive. Due to its interesting performance facilities, this method is suitable for routine analyses of water samples with a high concentration of contaminants, such as illegal spills from production plants or consumers, to ensure environmental safety at a low price. The method was also used to evaluate the stability of TBZ, 4-*t*OP and CPF in several situations (heated and stored in a fridge). The contamination of several waste and sewerage waters because of agriculture-related activity was determined.

Conflict of interest disclosure

The authors declare that they have no financial/commercial conflicts of interest.

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References

1 F. M. Fishel, *Pest Management and Pesticides: A Historical Early Uses of Pesticides and Other Insect-Vectored Diseases*, **PI219**, IFAS Extension, University of Florida, USA, 2013. Available at: http://edis.ifas.ufl.edu/pdffiles/PI/PI21900.pdf (accessed: 28/11/2014).
2 M. C. R. Alavanja, *Rev. Environ. Health*, 2010, **24**, 303–309.
3 G. G. Ying, B. Williams and R. Kookana, *Environ. Int.*, 2002, **28**, 215–226.
4 J. L. Suen, C. H. Hung, H. S. Yu and S. K. Huang, *Kaohsiung J. Med. Sci.*, 2012, **28**, S43–S48.
5 U.S. Environmental Protection Agency, *Pesticides*, Washington, DC, USA, 2014. Available at: http://www.epa.gov/pesticides/ (accessed: 28/11/2014).
6 M. H. Montforts, *Integr. Environ. Assess. Manage.*, 2006, **2**, 13–21.
7 A. Sengupta, J. M. Lyons, D. J. Smith, J. E. Drewes, S. A. Snyder, A. Heil and K. A. Maruya, *Environ. Toxicol. Chem.*, 2014, **33**, 350–358.

8 W. Körner, U. Bolz, W. Süssmuth, G. Hiller, W. Schuller, V. Hanf and H. Hagenmaier, *Chemosphere*, 2009, **40**, 1131–1142.

9 J. H. Ross, J. H. Driver, R. C. Cochran, T. Thongsinthusak and R. I. Krieger, *Ann. Occup. Hyg.*, 2001, **45**, S5–S17.

10 E. Fattore, R. Fanelli and C. La Vecchia, *J. Epidemiol. Commun. Health.*, 2002, **56**, 831–832.

11 F. Ferrara, F. Fabietti, M. Delise, A. P. Bocca and E. Funari, *Environ. Sci. Technol.*, 2001, **35**, 3109–3112.

12 Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 86/280/EEC, *OJE*U, 2008, **L 348**, 84–97.

13 U.S. Environmental Protection Agency, *Monitoring and Assessing Water Quality*, Washington, DC, USA, 2013. Available at: http://water.epa.gov/type/watersheds/monitoring/ (accessed: 28/11/2014).

14 *The pesticide manual: a world compendium*, ed. C. D. S. Tomlin, 15th edn, British Crop Production Council, Alton, UK, 2003.

15 European Commission, Health & Consumer Protection Directorate-General, *Review report for the active substance chlorpyrifos*, SANCO/3059/99 - rev 1.5, 2005. Available at: http://ec.europa.eu/food/plant/protection/evaluation/existactive/list_chlorpyrifos.pdf (accessed: 28/11/2014).

16 UK Environment Agency, Environmental Risk Evaluation Report: 4-tert-Octylphenol, Bristol, UK, 2005. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/290844/scho0405biyz-e-e.pdf (accessed: 28/11/2014).

17 H. C. Liang, N. Bilon and M. T. Hay, *Water Environ. Res.*, 2013, **85**, 2114–2138.

18 Z. Sosa-Ferrera, *Biomed. Res.*, 2013, **2013**, 1–23.

19 M. Petrović, E. Eljarrat, M. J. López de Alda and D. Barceló, *TrAC, Trends Anal. Chem.*, 2001, **20**, 637–648.

20 V. Belenguer, F. Martinez-Capel, A. Masiá and Y. Picó, *J. Hazard. Mater.*, 2014, **265**, 271–279.

21 J. E. Loyo-Rosales, C. P. Rice and A. Torrents, *Chemosphere*, 2007, **68**, 2118–2127.

22 M. Petrovic and D. Barceló, *J. AOAC Int.*, 2001, **84**, 1074–1085.

23 M. Petrovic and D. Barceló, *J. Mass Spectrom.*, 2001, **36**, 1173–1185.

24 M. Petrović, A. D. F. Ventura and D. Barceló, *Anal. Chem.*, 2001, **73**, 5886–5895.

25 U. Kotowska, J. Kapelewska and J. Sturgulewska, *Environ. Sci. Pollut. Res.*, 2014, **21**, 660–673.

26 B. Chen, B. Jin, R. Jiang, L. Xie, Y. Lin, W. Feng and G. Ouyang, *Anal. Methods*, 2014, **6**, 1743–1752.

27 F. Oliveira Silvério, J. G. Siqueira Silva, M. C. Santana Aguiar, A. P. Cacique and G. Paulino de Pinho, *Quim. Nova*, 2012, **35**, 2052–2056.

28 L. Brossa, E. Pocurull, F. Borrull and R. M. Marcé, *Chromatographia*, 2002, **56**, 573–576.

29 I. Cruceru, A. Florescu, I. A. Badea and L. Vladescu, *Environ. Monit. Assess.*, 2012, **184**, 6061–6070.

30 M. Asensio-Ramos, J. Hernández-Borges, G. González-Hernández and M. A. Rodríguez-Delgado, *Electrophoresis*, 2012, **33**, 2184–2191.

31 M. Tobiszewski and J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2012, **35**, 67–73.

32 B. Beltrán-Martinavarro, *Doctoral Thesis: Determinació cromatogràfica de melamina en mostres alimentàries i fisiològiques*, University Jaume I, 2013.

33 B. Beltrán-Martinavarro, J. Peris-Vicente, S. Marco-Peiró, J. Esteve-Romero, M. Rambla-Alegre and S. Carda-Broch, *Analyst*, 2012, **137**, 269–274.

34 M. Rambla-Alegre, J. Peris-Vicente, J. Esteve-Romero and S. Carda-Broch, *Food Chem.*, 2010, **123**, 1294–1302.

35 B. Beltrán-Martinavarro, J. Peris-Vicente, M. Rambla-Alegre, S. Marco-Peiró, J. Esteve-Romero and S. Carda-Broch, *J. AOAC Int.*, 2013, **96**, 870–874.

36 M. L. Chin-Chen, M. Rambla-Alegre, A. Durgbanshi, D. Bose, S. K. Mourya, J. Esteve-Romero and S. Carda-Broch, *Int. J. Anal. Chem.*, 2012, **2012**, 809513.

37 J. Peris-Vicente, S. Carda-Broch, J. Esteve-Romero, M. Rambla-Alegre, D. Bose, B. Beltrán-Martinavarro, S. Marco-Peiró, A. Martinavarro-Domínguez, E. Ochoa-Aranda and M. L. Chin-Chen, *Bioanalysis*, 2013, **5**, 481–194.

38 J. R. Torres-Lapasio, *Michrom Software*, Marcel-Dekker, NY, USA, 2000.

39 I. Casas-Breva, J. Peris-Vicente, M. Rambla-Alegre, S. Carda-Broch and J. Esteve-Romero, *Analyst*, 2012, **137**, 4327–4334.

40 M. Rambla-Alegre, J. Peris-Vicente, S. Marco-Peiró, B. Beltrán-Martinavarro and J. Esteve-Romero, *Talanta*, 2010, **81**, 894–900.

41 E. Ochoa Aranda, J. Esteve-Romero, M. Rambla-Alegre, J. Peris-Vicente and D. Bose, *Talanta*, 2011, **84**, 314–318.

42 N. Agrawal, J. Esteve-Romero, D. Bose, N. P. Dubey, J. Peris-Vicente and S. Carda-Broch, *J. Chromatogr., B: Biomed. Appl.*, 2014, **965**, 142–149.

43 J. J. Fernández-Navarro, M. C. García-Álvarez-Coque and M. J. Ruiz-Ángel, *J. Chromatogr., A*, 2011, **1218**, 398–407.

44 G. Ramis-Ramos and M. A. García-Álvarez-Coque, *Quimiometría*, Síntesis, Madrid, Spain, 2001.