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HIABENDAZOLE, 4-TH SEVERAGE WATER BY CHRON
ano <sup>1</sup> , Diego Kassuha <sup>2</sup> , Ju ep Esteve-Romero <sup>1</sup> Química Física i Analítica, ea y Farmacología, Faculta niversidad Católica de Cuy
uthor e-mail: vicentej@uji.
i liquid chromatographic the pesticides thiabendazole alations, the 4-tert-octylph large extraction steps usin thaminants were eluted with decyl sulfate - 6 % 1-penta r isocratic mode. This op siders the retention factor, hases. The detection was of ated in terms of: specificite etection and quantification (95.2 - 102.9 %) and preci- (at least 14 days) was stud
lysis time, then useful for e analysis of wastewater erage water belonging to an already reliable method.
nenol; Micellar; Pesticide;

#### ANALYSIS OF T ERT-OCTYLPHENOL AND CHLORPYRIFOS IN **Y DIRECT INJECTION - MICELLAR LIQUID** WASTE AND MATOGRAPHY

an Peris-Vicente<sup>1</sup>\*, Pasqual Roca-Genovés<sup>1</sup>, Samuel Ricard Romero-Ca Carda-Broch<sup>1</sup>, Jose 

<sup>1</sup>Departament de C ESTCE, Universitat Jaume I, 12074 Castelló, Spain

<sup>2</sup> *Química Biológic* ad de Ciencias de la Alimentación, Bioquímicas y

Farmacéuticas, Un o, San Juan, Argentina

es - Tel: +34964728099 - Fax: +34 964728066 \*Corresponding au 

Abstract 

A micellar method has been developed for the simultaneous quantification of th e and chlorpyrifos, as well as an alkylphenol included in pesticide formu henol, in water. The sample is filtered and directly injected, avoiding g toxic solvents, and then expediting the experimental procedure. The con hout interferences in < 17 min, using a mobile phase of 0.15 M sodium doc nol buffered at pH 3, running through a C18 column at 1 mL min<sup>-1</sup> under ptimal mobile phase was selected using a statistical approach, that cons efficiency and peak shape of the analytes measured in only few mobile pl carried out by absorbance at 220 nm. The method was ty, calibration range (0.5 - 10 mg L<sup>-1</sup>), linearity ( $r^2$ successfully valida  $(0.2 - 0.3; \text{ and } 0.5 - 0.8 \text{ mg L}^{-1}, \text{ respectively})$ , intra- and >0.994), limit of de ision (< 8.3 %), ruggedness (< 9.3 %). The stability in interday accuracy storage conditions died. The method was safe, inexpensive, low pollutant and with short anal routine analysis of these samples. Finally, the method was applied to the from fruit-processing industry, wastewater treatment plants, and in sewe the Castelló area (Spain). The results were similar to those obtained by a 

Keywords: Alkylph Validation; Water. 

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#### **1. Introduction**

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

These hazardous compounds are incorporated to agricultural and food-processing plant waste and municipal sewerage water, which are furthermore 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. Thus, some quantity of pesticides and APEs can remain in the effluent water discharged to the river<sup>7,8</sup>. The occurrence of these contaminants in natural water causes a serious damage to local flora and fauna<sup>2,3</sup>. The population is also directly exposed to this contamination by accidental inhalation, dermal and oral contact with polluted water<sup>3,9</sup>, and, through the food chain, by consumption of edible tissue of animals and plants grown with contaminated water<sup>10,11</sup>. Actually, these chemicals are cataloged as "Emerging Pollutants", hazardous compounds that have to be controlled and regulated due its potential environmental and health hazards. European Union, through "EU Water Framework Directive"<sup>12</sup> and the US Environmental Protection Agency<sup>13</sup> have implemented programs and policies to monitor these compounds in surface water. 

Thiabendazole (TBZ) is a fungicide and antiparasitic, largely used as a post-harvest preservative in various fruits and vegetables. Thiabendazole health effects include red blood cells, liver and thyroid damages. It is even carcinogenic at high concentrations<sup>14</sup>. Chlorpyrifos (CPF) is an insecticide, extensively pre-harvest utilized in agriculture to protect crops as cotton, corn, almonds, orange and apples, and in household to protect ornamental plants, lawn, pets and wood objects<sup>5</sup>. CPF is quite toxic and cause diseases and disrupting effect on the nervous system by short term contact<sup>15</sup>. The short APE 4-tert-octylphenol (4-tOP) is a product of the degradation by aerobic hydrolysis of long APEs spiked in formulations<sup>16</sup>. APEs show endocrine disruption effects, altering the hormonal system. Besides, 4-tOP shows higher toxicity and bioaccumulation than their long APE precursors<sup>3,4</sup>. These compounds are largely used in the Castelló area, due to its strong fruit agriculture and fruit-processing industry, introducing a high risk of water contamination. Thus, the 

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69 monitoring of TBZ, CPF and 4-tOP in waste and sewerage water is required to protect population70 health and the environment.

A high amount of analytical methodologies has been developed to detect pesticides<sup>17</sup> and alkylphenols<sup>18,19</sup> in several kinds 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<sup>20</sup> and APEs<sup>21-24</sup> in water samples, although GC-MS remains being used<sup>25,26</sup>. However, mass spectrometry is an expensive instrumentation and then analyses are sold at a too high price. HPLC coupled with UV-Visible absorbance (DAD) is an economic alternative and has been shown as successfully in several reports<sup>27-30</sup>. Waste and sewerage water usually contain suspended sludge and oily compounds, requiring a sample preparation to avoid the introduction of harmful substances in the chromatographic system. The experimental procedure involves tedious and time consuming clean-up steps, as solid/liquid<sup>20,21,22,24,28,30</sup> and liquid/liquid<sup>27,29</sup> extraction, improving the 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 performed to develop analytical methodologies avoiding these problems<sup>31</sup>. 

Micellar liquid chromatography, using hybrid mobile phases containing sodium dodecyl sulfate (SDS) as surfactant and a short-chain alcohol, has been demonstrated as an interesting alternative to hydroorganic-RP-HPLC<sup>32</sup>. Micellar solutions are able to solubilize compound within a high range of polarity. Therefore, samples with hydrophobic compounds can be directly injected, without risk of column damaging. Moreover, the surfactant monomer coat on the external layer of the stationary phase, changing its characteristics. The analyte is partitioned between three environments (stationary phase, mobile phase and micelle), thus improving the versatility of MLC<sup>33</sup>. 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 obtained in few several mobile phases, 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<sup>34</sup>. Micellar liquid chromatography has been previously proposed to detect chemical pollutants in wastewater<sup>35</sup>, and the pesticide carbaryl<sup>36</sup> in environmental water. 

99 The aim of this work is to develop a rapid, easy-to-handle, inexpensive, environmentally 100 friendly and reliable method to detect the pesticides TBZ, CPF and the short APE 4-tOP in water 101 samples, in order to apply it to routine analysis. The features of MLC would be exploited to allow 102 the direct injection of the sample and resolve the mixture of the analytes in a short chromatographic 103 run. The method should be validated in terms of calibration, linearity, sensitivity, intra- and interday

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104 accuracy and precision, ruggedness and stability to prove its reliability<sup>37</sup>. Finally, the developed 105 analytical method was used to quantify the analytes in WWPT influent and effluent, industrial 106 waste from fruit-processing industry, and sewerage water samples, collected at several points of the 107 Castelló area. The results ought to be compared with those obtained used by a reference method 108 based on LC-MS.

## 110 2. Material and methods

## 112 2.1 Chemicals and equipments

Standards of TBZ, CPF and 4-tOP (purity > 99.0 %), were purchased from Dr. Ehrenstorfer-Schäfers (Augsburg, Germany). Structure and main physicochemical characteristics are shown in Table 1. The characteristics of these compounds are in Table 1. SDS (purity > 99.0 %), methanol. 1-butanol, 1-pentanol (HPLC grade) were 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<sub>4</sub>), both HPLC grade, were bought 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 the aqueous solutions.

## *2.2 Preparation of solutions and mobile phases*

127 The stock solutions of the pesticides were prepared weighing a portion and dissolving in 128 methanol, in order to obtain concentrations of 100  $\mu$ g mL<sup>-1</sup>. Working solutions were prepared by 129 diluting these stock solutions in methanol to reach the desired concentration. All solutions were 130 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. The adequate volume of TEA or EMIMBF<sub>4</sub> was added, then the pH was adjusted by adding drops of HCl or NaOH solutions to reach the desired value. Furthermore, the adequate volume of short-chain alcohol was added, the solution was filled up to the final volume with ultrapure water, ultrasonicated and filtered.

All 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 in a Mettler-Toledo analytical balance (Greifensee, Switerland). A GLP 22 potentiometer (Crison, Barcelona) equipped with a combined Ag/AgCl/glass electrode used to measure pH values. The ultrasonication of 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 acquired by a personal computer connected to the chromatograph by means of an Agilent Chemstation version B.01.01. The chromatographic parameters retention time (t<sub>R</sub>, min), peak area (A, arbitrary units), dead time (t<sub>0</sub>, min), retention factor (k), efficiency (N, theoretical plates) and asymmetry (B/A) were obtained from the registered chromatograms using the Michrom software<sup>38</sup>. The meaning of these chromatographic parameters can be found in<sup>39</sup>. 

#### 2.4 Chromatographic conditions

The stationary phase was coated on a Kromasil C18 column (125 x 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<sub>2</sub>PO<sub>4</sub> at pH 3, running under isocratic mode at 1 mL min<sup>-1</sup> at room temperature. Injection volume was 20 µL and the absorbance detection wavelength was set at 220 nm. The special care with the chromatographic system when dealing with micellar mobile phases can be seen in<sup>40</sup>. Under these conditions, the column has a lifespan of nearly 1000 injections<sup>40</sup>. 

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 along the February-May period in several places where the presence of TBZ, CPF or 4-tOP is suspected: influent and effluent of WWPT, fruit-processing plant wastewater and sewerage water (Table 2). The samples were kept in a fridge protected from light (amber glass) until analysis. 

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

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## 3. Results and discussion

*3.1 Optimization of the chromatographic conditions* 

The column, injection volume and flow rate were taken as the most usual conditions in MLC, whereas the composition of the mobile phase and the detection condition were optimized. A standard solution containing 2  $\mu$ g mL<sup>-1</sup> of TBZ, CPF and 4-tOP was used for the optimization.

3.1.1 Optimization of the pH

184 The pH was selected in the working range of the column (1.5 - 7.5). The mobile phase was185 buffered to avoid variation of pH when the injected sample the mobile phase flow.

Three mobile phases as described in section 2.4, but buffered to 3, 5 and 7 were 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-tOP, the peak shape was comparable at the three pH. As consequently, pH 3 was selected for the analyses.

## 3.1.2 Selection of the organic modifier

According to the strong hydrophobicity of 4-tOP and CPF, a pure SDS solution would be unable to elute them from a C18 column in a reasonable retention time<sup>41</sup>. Therefore, SDS/1propanol 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<sup>32</sup>. In the three mobile phases, the elution order was:  $t_R(TBZ) < t_R(4$ tOP)  $< t_R(CPF)$ , 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

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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-tOP and CPF show a bending behavior face to SDS, the retention factor and the efficiency decrease at higher SDS concentration. As expected, the elution power and the peak shape are increased at upper 1-pentanol amount.

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 ( $\varphi$ ) concentrations of the mobile phase are related by the following equation<sup>32</sup>:

218  
$$k = \frac{K_{AS} \frac{1}{1 + K_{AD} \varphi}}{1 + K_{AM} \frac{1 + K_{MD} \varphi}{1 + K_{AD} \varphi} [M]}$$

The constants signify partition coefficients between phases. Their meaning can be found in:  $K_{AS}$ , partition constant between stationary phase and aqueous environment;  $K_{AM}$ , partition coefficient between the micelle and the aqueous environment, and  $K_{AD}$  and  $K_{MD}$ , 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<sup>42</sup>. Another equation allows to model the peak shape (N and B/A) at several SDS/1-pentanol concentrations<sup>32</sup>.

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

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) at the minimum analysis time ( < 20 min). A solution containing 2 µg mL<sup>-1</sup> of each studied pollutant was analyzed. The experimental chromatographic parameters ( $t_R$ ; N and B/A) were: TBZ (3.82 min; 1490; 0.97), 4-tOP (7.43 min; 1340; 1.08) and CPF (14.16 min; 1110 and 1.06). The obtained chromatogram can be seen in Figure 1A. As predicted, the mixture was completely resolved in an adequate time (< 17 min), assessing the high specificity of the method. The errors in the expected retention factors were below 6 %.

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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<sup>43</sup>. This avoids their interaction with the column, preventing the formation of a tailing and improving the peak shape. Therefore, two mobile phases containing 0.5 % of TEA and EMITBF<sub>4</sub> were tested. In both cases, the retention factors of the analytes increases without improvement of the peak shape, if compared with the mobile phase selected in 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-tOP and CPF was analyzed through the previously selected optimized conditions, at wavelengths ranging from 200 and 300 nm by measuring each 10 nm. Thus, we obtain 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 a 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 the time analysis and the simplification of the experimental procedure. This is possible because of the possibility of direct injection, which allows the elimination of intermediate extraction steps. Besides, the elution of the analytes is performed in < 17 min using isocratic mode, due to the use of micellar mobile phases. Thus, the stabilization time between two successive injections, required in gradient, 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 chemical, and the optimized mobile phase uses a less amount of organic solvents (6 % 1-pentanol), than typically used in hydroorganic HPLC (up to 100 %).

The analysis can be performed at low prices, because only the method requires basic
chromatographic instrumentation is required and low amount of inexpensive reagent is used.
Besides, the analysis of a large amount of samples per day is possible.

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All these features make the method available even for laboratories with low economic power, allowing to sell these analyses at low price, and then extremely useful for 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 their performance. The validation parameters were: calibration range, linearity, intra- and interday accuracy and precision, ruggedness and stability<sup>37</sup>. The whole calibration was performed in ultrapure water.

3.2.1 Calibration and sensitivity

3.2.2 Accuracy and precision

For calibration purposes, eight solutions containing increasing concentrations of TBZ, 4-tOP and CPF in the range 0.5-10 µg mL<sup>-1</sup> were analyzed by triplicate. The slope, y-intercept, regression coefficients and determination coefficients were obtained by plotting the peak are (average of the three measurements) v.s. the concentration using the least-square linear regression method. The study was repeated five days over a 3-months period, by preparing each time the standard solutions. The calibration curves were taken as the average values of these five regression curves. Results are shown in Table 2. An excellent linearity (r > 0.997 and  $r^2 > 0.994$ ) was found for the three contaminants in the range LOO -  $10 \mu g m L^{-1}$  (see below). 

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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. LOD was taken by visual appreciation following the 3 signal-to-noise ratio criterion, and was the concentration value providing a signal 3 times the baseline noise. The baseline noise was measured for each analyte, by analyzing a blank and at measuring the width of the baseline at the corresponding retention time<sup>37</sup>. The LOQ was taken as the lowest point of the calibration curve with a precision < 20 % and accuracy between 80 - and 120 % (see section 3.2.2)<sup>37</sup>. 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 sewerage water.

 309 The intra- and inter-day accuracy and precision were determinate at three concentration 310 levels (1; 2 and 5  $\mu$ g mL<sup>-1</sup>). The intra-day accuracy was calculated as the ratio concentration

provided by the method (average value of 6 analysis taken the same day)/true value. The intraday precision was the RSD of the peak area obtained by six analysis on the same day. Interday accuracy was calculated as the average of the intraday values obtained at five different days over a 3-months period, and using renewed solutions. Interday precision was measured as the RSD of the peak area of days over a 3-months 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-tOP and CPF in water, assessing the reliability of the quantitative data.</p>

 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 concentration, 1-pentanol amount, pH and flow rate. To study the influence of a determinate condition, a standard solution containing 2  $\mu$ g mL<sup>-1</sup> of each analyte was analyzed at three mobile phases: at its optimal value, slightly under and slightly over, maintaining the other constant. Thus, the influence of each parameter was separately studied. The considered ranges were: 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<sup>-1</sup>) by triplicate. The RSD of the measured retention time and peak area was 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-tOP and CPF.

 3.2.4 Stability

The stability of the analytes in water was studied at  $+60^{\circ}$ C and at fridge storage conditions (+4°C in darkness). Although 60°C are rarely reached in a real situation, the results would provide interesting information about the thermostability of the analytes. In both cases, a solution containing 1 µg mL<sup>-1</sup> of TBZ, 4-tOP and CPF was used.

The pollutant standard solution was heated at 60°C in a water bath. An aliquot was analyzed each 20 min during a 3-h period. The peak area corresponding to the contaminants remain nearly constant. Therefore, TBZ, 4-tOP and CPF are quite thermostable and can not be removed by heating.

The standard solution was kept in a fridge, then at +4°C and in darkness. Daily, an aliquot was analyzed, and no significant diminution in the peak area until 14 days. Therefore, a water

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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 provided to us by FACSA. The samples
were collected from of sewerage, industrial waste, and influent and effluent WWPT water from
several towns located in the Castelló area (Spain), where the occurrence of TBZ, 4-tOP and CPF is
suspected. We analyzed the water samples at a maximum of three days after received. 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-tOP and CPF can be seen in Table 4. Despite of the presence of
suspended sludge in several samples, neither obstruction nor damaging were noticed in the column,
needle or tubes. Figure 1B shows the chromatogram obtained by analyzing the sample 13,
indicating the other water contaminants elute fat 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 v.s. those obtained by LC-MS, using the least-square linear regression <sup>44</sup> .
Only the samples providing reliable concentration values (over LOQ) were taken. The obtained
curve was:
$[MLC] = (1.13 \pm 0.08) [LC-MS] + (-0.09 \pm 0.08) r^2 = 0.96$ 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-test tailing, the tabulated value t-student
was 2.26 ( $t_{0.05;9; 2tails}$ ). Thus, the confidence intervals were [0.96 to 1.34] and [-0.28 to 0.13] for
slope and y-intercept, respectively, thus the null hypothesis was accepted. Consequently, the results
obtained by our MLC method were close to those FACSA obtained by LC-MS. Although the
sensitivity is lower, the analysis can be performed at lower price. Then the MLC methods can be
applied to samples with a high contamination degree.
CPF has only been detected in one sample, indicating that it remains in crops and sludge,

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3.3 Analysis of real samples from sewerage and wastewate The developed method was applied to the analysis were collected from of sewerage, industrial waste, and ir several towns located in the Castelló area (Spain), where the suspected. We analyzed the water samples at a maximum FACSA analyzed the samples using its own standardized reasons, FACSA has not provided us the characteristics of and the content of TBZ, 4-tOP and CPF can be seen suspended sludge in several samples, neither obstruction no needle or tubes. Figure 1B shows the chromatogram indicating the other water contaminants elute fat from the re-The concordance of the results obtained by the two data obtained by MLC v.s. those obtained by LC-MS, us Only the samples providing reliable concentration values curve was:  $[MLC] = (1.13 \pm 0.08) [LC-MS] + (-0.09 \pm 0.08) r$ The two values show an adequate correlation. A sta assess the equivalence of the two values of each pair: Null = 0. Considering a significance level of  $\alpha$  = 0.05 and a two-was 2.26 ( $t_{0.05:9:2tails}$ ). Thus, the confidence intervals were slope and y-intercept, respectively, thus the null hypothesis obtained by our MLC method were close to those FAC sensitivity is lower, the analysis can be performed at lowe applied to samples with a high contamination degree. CPF has only been detected in one sample, indicate rather than reaching water. We can see that TBZ occurs in almost all samples, due to its extended use. In fact, even the sewerage not receiving agricultural waters contain TBZ. The contamination of the sewerage water receiving wastewater from fruit production is slightly above, indicating that the pesticides are moderately applied to crops and arrives diluted to the sewerage. 

Wastewater from fruit-processing plants show a moderate/low concentration of TBZ and 4tOP, indicating that these industries partially purge the wastewater before discharge. The influent samples from WWPT show higher concentrations than effluent, assessing that the analytes are removed from wastewater and ensure the validity of the water purification treatment.

384 4. Conclusions

The obtained data indicate that micellar liquid chromatography can be used in to analyze TBZ, 4-tOP 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, assessing its reliability. Besides, the method is safer for the operator and environmental friendly, thus making it more attractive. Due to its interesting performance facilities, this method is suitable for routine analysis of water samples with high concentration of contaminants, as illegal spills from production plants or consumers, to ensure environmental safety at low price. The method was used to evaluate the stability of TBZ, 4-tOP and CPF in several situations (heated and stored in a fridge). The contamination of several waste and sewerage waters because of the agriculture-related activity was overseen.

- - **5. Acknowledgments**

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## 406 6. Conflict of interest disclosure

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

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496	FIGURE CAPTIONS							
497	Figure 1. Chromatogram obtained by the analysis of: A) a mixture of 2 mg L <sup>-1</sup> of TBZ, 4-tOP and							
498	CPF and B) water sample 13 collected from the wastewater collector basin in the fruit-processing							
499	plant Invicto, Villarreal, Spain. Chromatographic conditions: C18 column, mobile phase 0.15 M							
500	SDS - 6 % 1-pentanol - pH 3; detection at 220 nm.							
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Compound	Structure	рКа	Charge at pH = 3	logP <sub>o/w</sub>
Thiabendazole <sup>15</sup>		4.73/12.00	+ 1	1.62
4-tert- octylphenol <sup>3</sup>	H <sub>3</sub> C CH <sub>3</sub> H <sub>3</sub> C CH <sub>3</sub> CH <sub>3</sub>	10.7	0	4.12
Chlorpyrifos <sup>17</sup>		Not applicable	0	4.70

Table 1. Structure and physicochemical parameters of the analytes.

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59 60 **Table 2**. Calibration and sensitivity parameters of the studied pollutants.

Compound	Slope	Intercept	r	$r^2$	LOD	LOQ
Thiabendazole	$0.8 \pm 0.1$	$-0.05 \pm 0.06$	0.997	0.9946	0.20	0.5
4-tert-octylphenol	$0.60\pm0.03$	$-0.04 \pm 0.03$	0.998	0.9966	0.25	0.6
Chlorpyrifos	$0.42 \pm 0.02$	$-0.03 \pm 0.06$	0.9993	0.9993	0.30	0.8

Slope and y-intercept: average value  $\pm$  standard deviation, Concentrations in  $\mu$ g mL<sup>-1</sup>; n = 5:

		Intra	-day <sup>a</sup>	Inter	-day <sup>b</sup>
Compound Thiabendazole 4-tert-octylphenol	Concentration	Accuracy	Precision	Accuracy	Precision
Compound	$(\mu g m L^{-1})$	(%)	(RSD, %)	(%)	(RSD, %)
	1	103.3	0.7	101.8	1.4
Thiabendazole	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

<b>Fable 3</b> .	Intra-	and	inter-d	ay a	accuracy	and	precision	for	TBZ,	4-OP	and	CPF.
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 $a_n = 6; b_n = 5$ 

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			Т	BZ		4-tOP		CPF
Origin of water sample	Sample	Location	MLC	LC-MS	MLC	LC-MS	MLC	LC-MS
	1	Vila-real	< 0.5	0.29	0.65	0.75	n.d.	0.24
Sewerage receiving	2	La Vilavella	< 0.5	0.41	n.d.	n.d.	n.d.	n.d.
agricultural	3	Betxí I	< 0.5	0.12	n.d.	0.1	n.d.	0.14
wastewater	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.
	6	Alcora	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sewerage not	7	Nules	< 0.5	0.04	n.d.	n.d.	n.d.	n.d.
receiving	8	Vila-real I	< 0.5	n.d.	n.d.	0.21	n.d.	n.d.
agricultural	9	Alcora	< 0.5	0.08	n.d.	0.14	n.d.	n.d.
wastewater	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	12	Real Export (Vila-real)	1.1	0.93	n.d.	n.d.	< 0.8	0.18
wastewater from a	13	Invicto (Vila-real)	0.9	0.85	n.d.	0.15	n.d.	0.12
fruit processing	14	Serifruit (Vila-real)	0.5	0.42	0.6	0.63	n.d	n.d.
plant	15	Eurococi (Betxí)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	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
Wastewater from	19	Effluent (Vora Riu)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
WWPT	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.

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**Table 4**. Concentrations (ug mL<sup>-1</sup>) of TBZ. 4-tOP and CPF detected in real water samples.

not detected (< LOD) n.a.

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Figure 1