Development and validation of a method to determine thiabendazole and o-phenylphenol in wastewater using micellar liquid chromatography-fluorescence detection

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Abstract

A micellar liquid chromatographic method to determine thiabendazole (TBZ) and o-phenylphenol in wastewater is described here. The sample was directly injected without any additional treatment other filtration. The pesticides were resolved in <11 min, using a mobile phase of 0.10 M SDS-6% 1-pentanol buffered at pH 3 running through a C18 column at 1 mL/min. The detection was performed by fluorescence at 305/360 and 245/345 nm excitation/emission wavelengths for TBZ and o-phenylphenol, respectively. The method was validated following the directives of the Validation and Peer Review of U.S. Environmental Protection Agency Chemical Methods of Analysis guidelines in terms of selectivity, quantitation range (0.01-0.02 to 2 mg/L), detection limit (0.005-0.008 mg/L), trueness (92.1-104.2%), precision (<13.9%), robustness (<6.6%), and stability under storage conditions. The procedure was applied to the screening of TBZ and o-phenylphenol in wastewater samples from citrus packing plants, agricultural gutters, urban sewage, as well as in influent and effluent wastewater treatment plants.

Keywords: Direct injection; Micellar; Pesticides; Validation; Wastewater

1. Introduction

Thiabenzadole (p*K*a = 4.7/12.0; log Po/*w* = 2.39; solubility in water = 28 mg/L) is a broad spectrum fungicide and antiparasitic [1, 2]. O-phenylphenol (OPP) (p*K*a = 9.4; log Po/*w* = 3.18; solubility in water = 700 mg/L) is a general biocide, with germicide, fungicide, bactericide, and nematicide activity, usually supplied as its sodium salt [2,3]. These pesticides are largely utilized in the agro-food industry as preservative agents. They are included in the post-harvest treatment of many fruits and vegetables commercialized for fresh consumption, to control fungal and mold diseases during storage and transportation, in order to maintain their nutraceutical properties and an attractive appearance. They are deposited on the surface to form a protective film by dipping, drenching or surface waxing [1,3]. They are also used in seed treatments to avoid spoilage [4].

Thiabendazole (TBZ) is used in pre-harvest agriculture and in urban gardening to protect bulbs and trees, by spraying on leaves, fruits and soil [1]. It has also been prescribed as an anthelmintic drug to treat infections caused by parasitic worms in human, livestock and pets [5]. O-phenylphenol (OPP) is the main active ingredient of many disinfectant formulations used to sterilize hard surfaces in agricultural, medical, commercial, institutional, residential and public access premises and equipment. It is also added as preservative to a broad range of materials, such as paints, stains, cement and wood products. It can be also found in cosmetics, as well as in household aerosols and cleaning products [3,6].

Because of their large variety of applications and massive use [1,3-6], TBZ and OPP can be found in wastewaters from diverse sources, such as urban sewage systems, agro-food industry discharges, and agricultural gutters [7, 8]. From there, they can diffuse into the atmosphere and may arrive to the environmental water [9]. They can attain remarkable levels in water [2] and in river sludge [1, 3]. Due to their high toxicity, they can damage the aquatic wildlife and the population, either by dermal contact or by inhalation [1, 3, 10, 11]. Wastewaters are purified in wastewater treatment plants (WWTP), but this process may be incomplete [12]. Besides, a large concentration of TBZ and OPP in influent waters reduces the capacity of the WWTP, and then the quality of the effluent stream [13].

Many municipal governments have launched actions to minimize the pollution level of the wastewaters in their area [14]. Therefore, maximum permitted levels have been fixed for spills and municipal sewage waters, and the effectiveness of the WWTP must be

97 evaluated. Besides, the agrofood industry has been requested to depurate the waste [7].
98 Therefore, TBZ and OPP must be continuously monitored in the different wastewaters.
99 We described in a previous paper a functional, reliable, easy-to-handle, rapid,
100 ecofriendly, and economic procedure to determine thiabendazole (TBZ) andOPP, together
101 with other fungicides, in wastewater by micellar liquid chromatography (MLC) DAD,
102 avoiding the major drawbacks of hydroorganic RP-HPLC [15]. In the present work, we
103 explore the possibility to detect TBZ and OPP in wastewater by fluorescence detection
104 (FLD), in order to increase the sensitivity and the selectivity. Indeed, TBZ [16] and OPP [17]
105 have been determined in water by HPLC-FLD. Besides, fluorescence is enhanced in
106 organized media [18]. The new MLC-FLD method should be validated following the
107 guidelines of the Validation and Peer Review of Environmental Protection Agency (EPA)
108 Chemical Methods of Analysis [19]. Finally, it has to be applied to wastewater samples from
109 several sources in the Spanish town of Villarreal (Castelló province). Therefore, the
110 sensitivity should be sufficient to detect the maximal tolerated concentrations in wastewaters
111 set by the Municipal Regulation of Villarreal (0.5 mg/L for a single spill and 0.1 mg/L as the
112 diary average concentration) [20].
113
114 **2. Materials and methods**
115
116 The reagents, apparatus, and the preparation of the mobile phases were the same as in
117 [15].
118
119 *2.1 Chromatographic conditions*
120
121 The chromatographic analyses were carried out using an Agilent Technologies Series
122 1100 (Palo Alto, CA,USA), equipped with an isocratic pump, a degasser, an autosampler, and
123 a fluorescence detector, connected to a computer. The software Chemstation version B.01.01
124 was used to control the instrument, as well as to acquire and process the signals.
125 The analytes were eluted using a hybrid micellar mobile phase of 0.10 M SDS/6% 1-
126 pentanol at pH 3 (fixed with 0.01 M phosphate buffer), running at 1 mL/min under isocratic
127 mode through a Kromasil (Sigma-Aldrich, Saint-Louis, MO, USA) C18 column (150 × 4.6
128 mm; particle size, 5 μm; pore size, 10 nm). The injection volume was 20 μL. The

fluorescence signal was registered using the following excitation/emission (nm) wavelengths program: 0-8.0 min, 305/360; and 8.0-11.0 min, 245/345.

2.2 Sample collection and processing

Wastewater samples (Table 1) were collected at several points of the Castelló province, in Spain, between February and May, 2015. The samples were stored in amber bottles in a fridge (+4ºC), and analyzed before 2 weeks.

The samples were thawed 30 min at room temperature before processing. Thereafter, an aliquot was filtered and directly injected.

3. Results and discussion

3.1 Optimization of the chromatographic conditions

The main chromatographic conditions (see Section 2.1) were taken from [15]. TBZ is positively monocharged and OPP is neutral, and both are moderately hydrophobic. Therefore, they would be adequately retained in a C18 column.

The SDS and 1-pentanol concentrations in the mobile phase and the detection conditions were optimized to determine TBZ and OPP.

160 **Table 1**. Origin, sampling way and date, and TBZ/OPP concentration (mg/L) of the studied samples (supplied
161 by FACSA, Castelló, Spain).

Origin	Nº	Date	Sampling	Place	TBZ	OPP
Wastewater collector basin from a fruit packing plant	1	07/02	Spot	Real Export (Villarreal)	1.09	0.78
	2	14/02	Spot	Serifruit (Villarreal)	0.78	1.1
	3	28/02	Spot	Invicto (Villarreal)	0.89	0.95
	4	28/02	Spot	Eurococi (Betxí)	0.09	0.51
Agricultural gutter	5	11/02	Continuous 08:00-18:00 h	Villarreal	0.31	n.d.
	6	24/02	Continuous 24 h	La Vilavella	0.44	n.d.
	7	26/02	Continuous 24 h	Alcora	0.51	n.d.
	8	27/03	Continuous 24 h	Betxí	0.24	n.d.
	9	13/05	Continuous 08:00-18:00 h	Onda	0.18	n.d.
Urban sewage water	10	05/03	Continuous 24 h	Nules I	n.d.	0.02
	11	15/04	Continuous 08:00-18:00 h	Nules II	0.01	0.05
	12	22/04	Continuous 08:00-18:00 h	Villarreal I	0.06	0.03
	13	13/05	Continuous 08:00-18:00 h	Villareal II	n.d.	0.06
	14	14/05	Continuous 08:00-18:00 h	Alcora	n.d.	n.d.
	15	27/05	Continuous 08:00-18:00 h	Onda I	n.d.	n.d.
	16	25/06	Spot	Onda II	0.05	0.07
Wastewater from the WWTP Vora Riu (Villarreal)	17	05/03	Spot	Influent	1.57	1.34
	18	05/03	Spot	Effluent	0.02	n.d.
Wastewater from the Joint WWTP OBVA (Onda/ Betxí/Vilareal/ Alquerías)	19	06/04	Spot	Influent	0.84	1.75
	20	12/06	Spot	Decanted influent	0.91	1.83
	21	06/04	Spot	Effluent	n.d.	n.d.
Wastewater from the WWTP Nules, La Vilavella	22	19/02	Spot	Influent	0.71	1.1
	23	19/02	Spot	Effluent	n.d.	n.d.

162 The places refer to several towns from the Castelló Province (Spain) - n.d. = not detected

3.1.1 Optimization of the SDS/1-pentanol concentration

The SDS/1-pentanol concentrations were optimized following the criterion of maximal resolution–minimum analysis time using an interpretative strategy [15, 21-23].

The influence of SDS and 1-pentanol on the elution strength of the mobile phase was examined using a full factorial experiment. The experimental retention factors of TBZ and OPP were measured by five mobile phases containing a combination of the minimum and maximum concentration of SDS (M) and 1-pentanol (%) recommended for MLC (0.05/2; 0.05/6; 0.15;2 and 0.15/6), and another one at the intermediate values (0.1/4) [15]. In the five cases, TBZ was eluted before OPP, according to its higher polarity, and their peak shapes were quite Gaussian for the five mobile phases. Both pesticides show a binding behavior with SDS-micelles, as their retention factor diminishes when the number of micelles increases. Besides, the analytes were less retained by increasing the proportion of 1-pentanol, as usual in MLC.

The retention factors and the modified selectivity were modelled from the concentration of SDS and 1-pentanol in the mobile phase. Thereafter, the most adequate values were selected by response surface methodology.

3.1.1.1 Modeling of the retention behaviour

The retention factor (k) was modeled using the following empirical equation, applicable to moderately hydrophobic compounds [21]:

$$1/k = c_0 + c_1\,[\mathrm{SDS}] + c_2\,\varphi + c_{12}\,[\mathrm{SDS}]\,\varphi \qquad (4.1)$$

where φ is the proportion of 1-pentanol and the c_i are experimental constants.

For each pesticide, the data obtained from the factorial experiment were processed to fit this equation by nonlinear least-squares regression (curve fitting) [22]. The concentrations of SDS and 1-pentanol were normalized from their ranges (0.05 - 0.15 M) and (2 - 6 %), respectively, to (-1; +1), in order to compare the obtained constants. According to the obtained multiple regression coefficients (R^2), this hyperbolic model provides accurate predictions. The adjusted equations were:

TBZ $1/k=(0.164\pm0.003)+(0.072\pm0.004)[SDS]+(0.037\pm0.004)\varphi+(0.017\pm0.004)[SDS]\varphi$ $R^2=0.998$ (Eq 4.2)

OPP $1/k=(0.105\pm0.003)+(0.044\pm0.003)[SDS]+(0.017\pm0.003)\varphi+(0.010\pm0.003)[SDS]\varphi$ $R^2=0.997$ (Eq 4.3)

The values of the constants and their corresponding standard deviation allow us to infer interesting statements about the retention behaviour of both pesticides. The constants c_1; c_2 and c_{12} have a low relative standard deviation and a positive value, that means the rising of SDS or 1-pentanol amount effectively provokes a significant diminishing of the retention. In addition, the augmentation of the elution strength caused by the increase of the SDS concentration is more intense at higher proportions of 1-pentanol, and *vice versa*. The most influent parameter was the concentration of SDS, followed by the proportion of 1-pentanol and, finally, their interaction. Otherwise, the theoretical retention time was higher for OPP than for TBZ in the overall studied ranges. These assertions coincide with that previously observed in the qualitative interpretation of the untreated experimental results obtained from the factorial experiment.

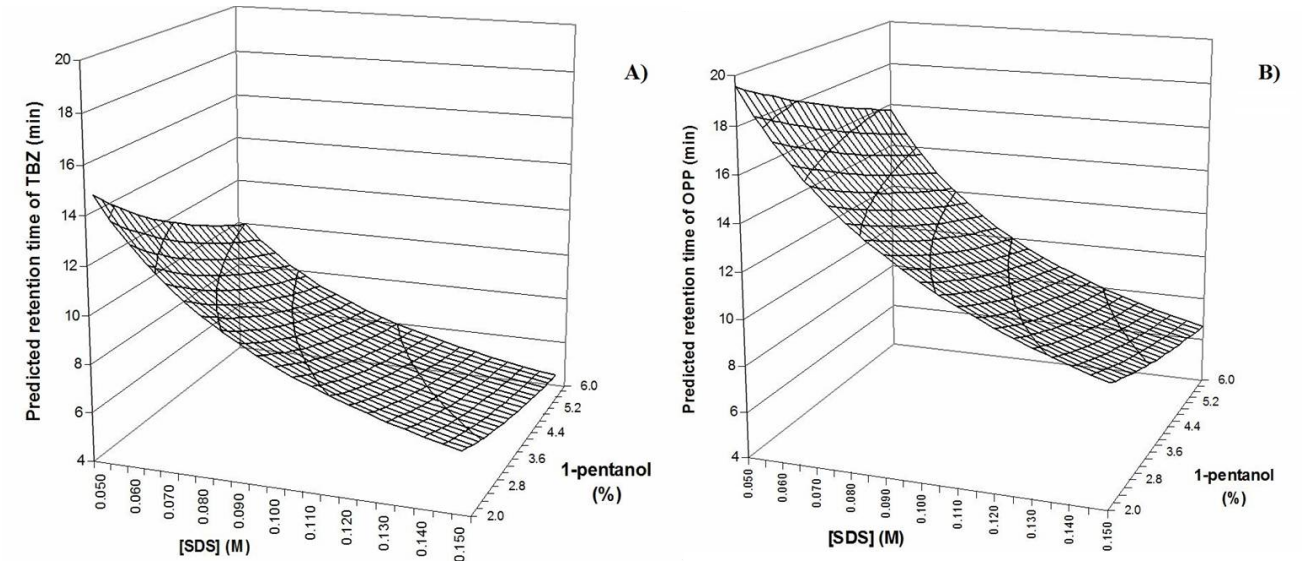

Figure 1. Predicted A) thiabendazole and B) o-phenylphenol retention time, *versus* the concentration of SDS and 1-pentanol.

3.1.1.2 Modeling of the chromatographic resolution

The selectivity (α) and the modified selectivity (*Z*) were modeled by the following equation [23]:

$$Z = 1\text{-}1/\alpha = 1\text{-}\ k(TBZ)/k(OPP) \quad (4.4)$$

$$Z = 1 - (0.164+0.072[SDS]+0.037\varphi+0.017[SDS]\varphi)/(0.105+0.044[SDS]+0.017\varphi+0.010[SDS]\varphi) \quad (4.5)$$

The theoretical modified selectivity can be seen in Figure 2.

3.1.1.3 Selection of the SDS/1-pentanol concentrations

SDS and 1-pentanol concentrations were selected considering their effect on the duration of the chromatographic run and the separation of both analytes. We can see that the maximal modified selectivity (0.3911) was achieved at 0.05 M SDS/6 % 1-pentanol, but with a too high analysis time, as OPP was eluted at nearly 15 min. At 6 % 1-pentanol, the modified selectivity was barely affected by the SDS concentration, while the retention times were significantly reduced. Finally, the selected mobile phase was an aqueous solution of 0.10 M SDS/6 % 1-pentanol phosphate-buffered at pH 3, which provides a theoretical modified selectivity of 0.389 and an expected retention time of 9.0 min for OPP. The error in the prediction of the retention factors was < 3.0 %.

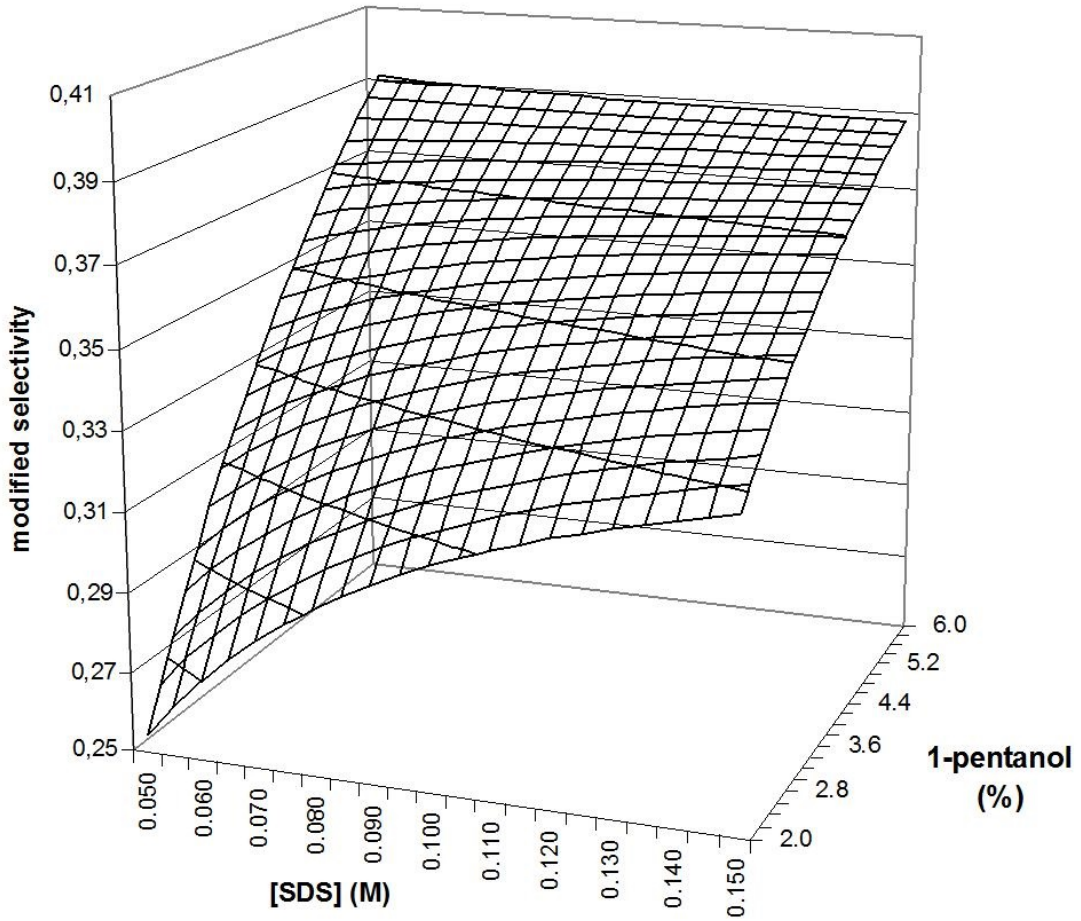

Figure 2. Predicted modified selectivity *versus* the concentration of SDS and 1-pentanol.

The selected mobile phase was an aqueous solution of 0.10 M SDS/6% 1-pentanol phosphate buffered at pH 3. Under these conditions, TBZ and OPP were eluted at 6.0 and 9.2 min, respectively (total analysis time, <11 min), with a quite Gaussian peak shape (Fig. 3A). The limited efficiency, as usual in MLC [21], was not a disadvantage, because the analytes do not overlap and no other peaks were detected.

3.1.2 Detection conditions

The detection conditions were optimized by a chromatographic analysis of both pesticides maintaining the other instrumental parameters at their previously selected values. The excitation and emission spectra were on-line measured using the fluorescence detector of the chromatograph, and the optimal excitation/emission wavelengths were selected by

254 iteration to maximize the S/N of the emitted luminescence. These values were 305/360 and
255 245/345 for TBZ and OPP, respectively.
256 The excitation/emission wavelengths were adjusted in time to quantify each pesticide
257 under its optimal conditions. Thus, the signal was monitored by the following program: 0-8.0;
258 305/360 (for TBZ) and 8.0-11.0; 245/345 nm (for OPP). No sudden variation of the baseline
259 signal was noticed at the change time, and the baseline noise was relatively narrow.
260
261 *3.2 Method validation*
262
263 The analytical procedure was within-laboratory validated following the directives of
264 the Validation and Peer Review of the EPA Chemical Methods of Analysis. The studied
265 parameters were instrument calibration, quantitation limits and range, detection limit, inter-
266 and intraday trueness and precision, robustness [19], and stability under storage conditions.
267 Seven solutions containing increasing concentrations of the pesticides (0.01–2 mg/L)
268 were analyzed (n = 3). For each pesticide, the peak area was correlated to the corresponding
269 concentration (mg/L) by least-square linear regression [23]. The resulting equations were:
270
271 TBZ A = (1524 ± 14)[TBZ] + (9 ± 4); r^2 = 0.9995; calibration range = 0.01-2.0 mg/L
272 OPP A = (525 ± 23)[OPP] + (1.0 ± 2.6); r^2 = 0.9998; calibration range = 0.02-2.0 mg/L
273
274 These calibration data refer to the concentration (mg/L) in the injected sample. As
275 neither dilution nor preconcentration steps were included in the analytical procedure, the
276 concentration in the sample equals that in the injected solution. Therefore, the quantitation
277 range is the same as the calibration range.
278

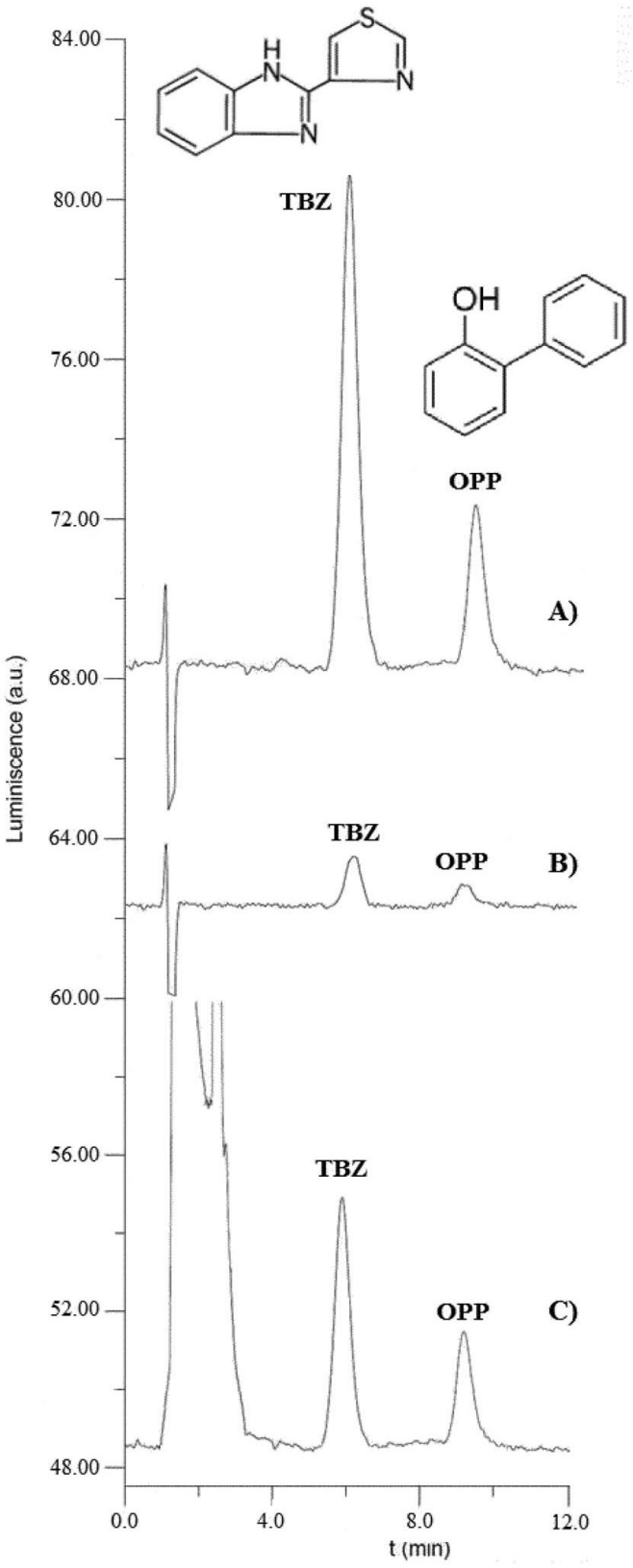

Figure 3. Chromatograms obtained by the analysis of a standard solution of TBZ and OPP (A) at 0.1 mg/L and (B) at their LLOQ, and (C) sample 16.

The lower (LLOQ) and upper (ULOQ) LOQ were taken as the smallest and the highest concentration of the calibration curve, respectively [23]. The LOD was calculated by the 3.3 criterion: TBZ, 0.005 mg/L and OPP, 0.008 mg/L. The chromatogram obtained by the analysis of a water sample spiked at their corresponding LLOQ can be seen in the Fig. 3B. The intra- and interday trueness and precision were studied as described in [15], at four concentrations: LLOQ, both regulatory levels (0.1 and 0.5 mg/L), and ULOQ. The method shows good recovery (92.1-104.2%) and low variability of the signals (<13.9%). The robustness was examined as indicated in [15], inside the following ranges: SDS concentration, 100 ± 5 mM; 1-pentanol proportion, 6.0 ± 0.2%; and pH, 3.0 ± 0.1. Insignificant variations of retention time (<6.5%) and peak area (<6.6%) were noticed.

A blank sample was spiked at 0.5 mg/L of TBZ and OPP and kept in a fridge as indicated in Section 2.2. During 2 weeks, an aliquot was daily taken and analyzed (n = 3), and no significant reduction of the peak area was observed.

The quantitation range includes the maximum permitted limits in discharges to the sewage system set by the municipal bylaw of Villarreal (Castelló, Spain) [20]. Besides, the sensitivity is able to detect moderate concentrations of TBZ and OPP in polluted water. Otherwise, the method provides reliable quantitative results, because of the high recovery, low variability, and the stability of the experimental response. In addition, the samples can be kept until 2 wk before analysis. Therefore, the developed procedure can be used to evaluate the compliance of the spills and the urban sewage with the regulation.

3.3 Analysis of wastewater samples

The concentrations of TBZ and OPP were measured in the wastewater samples described in the Table 1. In all cases, the matrix was barely retained, and the analytes were resolved without interferences. The chromatogram obtained from the analysis of the sample 16 is shown in Fig. 3C. Concentrations over the maximum permitted limit (0.1 mg/L) stated by the Municipal Regulation of Villarreal, were considered "high."

The collector basins from citrus packing plants showed an elevated quantity of both pesticides (except sample 4), as they are largely used for postharvest conservation purposes.

Agricultural gutters contained a significant concentration of TBZ, while OPP was not detected. Indeed, TBZ is preharvest applied in trees and fruits, and it is further dragged by

irrigation and rain water to the gutters. On the contrary, OPP is not directly used on crops. In urban wastewater, low concentrations of OPP and TBZ were detected. The higher occurrence of OPP was probably due to its higher domestic and public applications.

TBZ and OPP were found at high concentrations in influent WWTP streams. The occurrence of both pesticides is due to the presence of densely populated urban centers, strong industrial (for OPP) and fruit-agricultural activity in the area, both production (for TBZ) and processing (for TBZ and OPP).

Effluent streams contained no detectable quantities of both contaminants. Therefore, the purified water can be released to the environment.

4. Concluding remarks

The here-described procedure provides interesting advantages over that in [15]. First, the duration of the chromatographic run was significantly shortened from 14 to 11 min, because the present study was focused on two compounds. This permits the study of a larger number of samples per day, which is interesting for routine analysis. The use of FLD, instead of UV absorbance, also improved the quality of the method. The sensitivity was significantly augmented, as the detection limits were nearly 100 times lower (0.005-0.18 and 1.0-0.008 mg/L, for TBZ and OPP, respectively) and the baseline was narrower. This is a significant improvement, insomuch as the present method allows the detection of pollution at lower levels. Besides, the signals from the front of the chromatograms were lower, and less matrix compounds were detected, which enhances the specificity of the method. The other features were similar to [15]. Therefore, this MLC-FLD procedure is an interesting alternative for laboratories of public agencies, treatment plants, and the agro-food industry to monitor TBZ and OPP in wastewater samples, because of its interesting analytical performances. The results range are useful to detect the analyte at the levels that can be found in physiological fluids. The high linearity and recovery near 100% indicates that the values of concentration of paroxetine are near the true value. The low variability and high precision of the results obtained on different days are evident, which indicate the usefulness of the method. The developed method was found to be robust as the small deliberate changes in the method conditions did not have any significant effect on the chromatographic behavior of the analyte. The usefulness of the method has been assessed by the quantification of paroxetine in real

samples from paroxetine takers, no interference has been observed and the levels of paroxetine have been determined with adequate precision.

5. Acknowledgements and conflict of interest disclosure

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The authors have declared no conflict of interest.

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