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Use of micellar liquid chromatography for rapid monitoring of fungicides post harvest applied to citrus wastewater

Juan Peris-Vicente*, Ana Marzo-Mas, Pasqual Roca-Genovés,
Samuel Carda-Broch, Josep Esteve-Romero

Departamento de Química Física i Analítica, ESTCE, Universitat Jaume I, 12071 Castelló, Spain. E-mail: vicentej@ujj.es

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ABSTRACT

A method based on micellar liquid chromatography has been developed to simultaneously monitor four pesticides largely post-harvest applied to citrus: thiabendazole, pyrimethanil, *o*-phenylphenol and imazalil. Water samples were filtered and directly injected without other treatment, thus avoiding extraction steps. The composition of the mobile phase was optimized using a chemometrical approach to achieve an excellent resolution to 0.07 mol/L SDS/5%, V/V 1-pentanol buffered at pH 3. Mobile phase run through a C18 column at 1 mL/min at room temperature. The detection was performed by UV-Visible absorbance using a wavelength program: 0–10 min, 305 nm (for thiabendazole); 10–12; 265 nm (for pyrimethanil) and 12–18, 220 nm (*o*-phenylphenol and imazalil). The developed method was validated following the guidelines of the US Environmental Protection Agency in terms of: quantitation range, (0.5–4 to 15 µg/mL), linearity ($r^2 > 0.9995$), sensitivity (LOD, 0.18–1.4 µg/mL), precision (<9.2%), trueness (93.9%–103.7%), and ruggedness (<9.9%). It was found that the fungicides remain up to eight days in surface water at outdoor conditions. The method was used to screen the presence of the analytes in several waste water samples, and was proved to be useful in routine analysis.

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Introduction

The production of citrus and the related fruit processing industry have as strong importance in the Castellón area (Spain). In fact, the exportation of fruits has an important weight in its economy (Montoliu-Vidal, 2010). One of the problems of fruit trading is the spoilage of citrus during storage and transportation, caused by microorganism, fungi and insects. This reduces the economic yielding of the agricultural activity. To prevent this fruit decay, pesticides are post-harvest added to fruits (Burden and Wills, 1989). Thiabendazole (TBZ), pyrimethanil (PYR), *o*-phenylphenol (OPP), and imazalil (IMZ) are pesticides widely post-harvest applied by citrus traders and

fruit-processing industry to protect crops during storage and transportation, because of their broad spectrum and strong fungicide activity (US EPA 2015; Smilanick, 2011). TBZ and PYR are also pre-harvest used to protect the tree and citrus during growing against mold and fungi. They are applied to the soil or sprayed over crop fields (Smilanick, 2011; Picón-Zamora et al., 2000).

Because of their intensive use and persistence, pesticides represent an important source of contamination of environmental water, especially those near areas with strong fruit-related activity. These pesticides are highly toxic and potentially carcinogenic, and then represent a serious threat to the local flora and fauna (US EPA, 2015). The population is

* Corresponding author.

also exposed to these hazardous compounds by dermal contact, accidental ingestion, or inhalation of polluted water. Moreover, the main danger is the ingestion of animals of vegetables, which have been in contact with polluted water, due to because their high bioaccumulation in edible tissues of living organisms (Langenbach, 2013). For these reasons, the European Water Framework Directive recommends the implementation of actions to avoid these compounds arrive to underground and surface water (European Commission, 2008).

These pesticides are present in wastewater from agricultural field, drained by rain water (Langenbach, 2013), and in sewage water from fruit-processing plants. To avoid the pollution of environmental water by pesticides, sewerage and waste water are purified in wastewater treatment plants (WWTP) before discharging to the river streams. In order to evaluate the quality of waste and sewerage water, several local governments have implemented programs to periodically perform pesticide screening in water samples from their area. Moreover, the fruit processing industry has been requested to monitor these hazardous compounds in their own wastewater before throwing it, in order to reduce the environmental impact of their activity. The effectiveness of the WWTP treatment must also be evaluated by analyzing the influent and effluent flow (European Commission, 2008). Indeed, they must dispose of a reliable, easy-to-use and sensitive analytical method to simultaneously quantify thiabendazole, *o*-phenyl-phenol, pyrimethanyl and imazalil in water.

The preferred analytical methods to perform multiresidue pesticide analyses in wastewater are gas chromatography and liquid chromatography, coupled to mass spectrometry (Liang et al., 2014). However, this instrumentation is costly and requires expensive maintenance; thus, the analyses are sold at higher prices. In the current context of economic crisis, industries and government institutions are forced to reduce their budgets, and increasingly demand less expensive methods. Liquid chromatography can be coupled to affordable and reasonably selective detector, as UV-Visible, to detect TBZ (Cacho et al., 2009; Santaladchayakit and Srijaranai, 2012), PYR (Gao et al., 2012; Baggiani et al., 2007), OPP (Yu et al., 2001; Liu et al., 2014) and IMZ (Gao et al., 2012; Tian et al., 2012) in water and aqueous samples. However, the resolution of a pesticide mixture is normally performed using gradient-programmed mobile phases, complicating the screening of a large amount of samples (Cacho et al., 2009; Santaladchayakit and Srijaranai, 2012; Yu et al., 2001). Nevertheless, wastewater samples may contain sludge particles and oily compounds dispersed in water, which must be removed before analysis (Beltrán-Martínavarro et al., 2013). Thus, tedious and time-consuming clean-up steps must be introduced, as a liquid-liquid (Santaladchayakit and Srijaranai, 2012; Gao et al., 2012; Yu et al., 2001) or solid/liquid (Cacho et al., 2009; Baggiani et al., 2007; Liu et al., 2014; Tian et al., 2012) extraction. These steps require specific chemicals and materials, and increase the possibility of loss of analyte by low yielding or operator error.

Liquid chromatography using hybrid micellar mobile phases, containing sodium dodecyl sulfate (SDS) as surfactant and short-chain alcohol (to improve the elution power and the efficiency) as additive, is an interesting alternative to analyze contaminants in wastewater. The lipophilic environment inside the micelle allows the solubilization of hydrophobic compounds.

Therefore, after a simple filtration, the water sample can be directly injected in the chromatographic system, thus expediting the experimental procedure (Romero-Cano et al., 2015). The retention mechanism is different from hydroorganic reverse phase-high performance liquid chromatography (RP-HPLC), because the monomer surfactant modifies the nature of the stationary phase, and the analyte also can interact with the core of the micelles. Hence, compounds with dissimilar hydrophobicity can be resolved in the same run under isocratic conditions. The behavior of the analytes in micellar liquid chromatography (MLC) is highly steady and reproducible. The retention parameters can be accurately predicted at different SDS/alcohol concentration by means of a statistical treatment from the results obtained by testing only few mobile phases. Moreover, SDS solutions are more stable, less toxic, non-flammable, biodegradable, and uses less amount of organic solvent (up to 12.5%, V/V), in comparison to hydroorganic mobile phases used in HPLC (Peris-Vicente et al., 2014). MLC has been already used to detect carbamate pesticides in water (Gil-Agustí et al., 2002; Capella-Peiró et al., 2004; Chin-Chen et al., 2012).

The aim of the work is to develop an analytical procedure based on micellar liquid chromatography for the screening of TBZ, PYR, OPP and IMZ in water. The method must be simple, rapid, inexpensive, reliable and environmental friendly. The sample preparation must be simplified to facilitate the study of a large amount of samples, in order to apply it to routine analysis. The analytical procedure would be in-house validated by the Validation and Peer Review of U.S. Environmental Protection Agency (EPA) Chemical Methods of Analysis guideline in terms of selectivity, quantitation range, linearity, sensitivity, precision, trueness and ruggedness (The FEM Method Validation Team, 2005). The analytical method would be applied to evaluate the stability of the fungicides in outdoor conditions, and to detect the concentration of pesticides in sewage and WWTP treated water streams.

1. Materials and methods

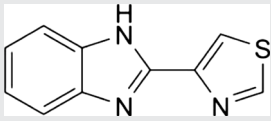
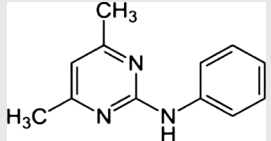
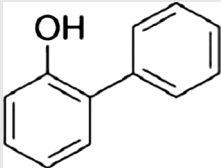
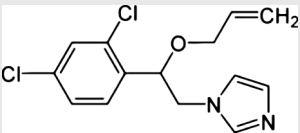
1.1. Reagents and solutions

The pesticides thiabendazole, pyrimethanyl, *o*-phenyl-phenol and imazalil (purity >99.9%) were purchased from Dr. Ehrerstorfer (Augsburg, Germany). The characteristics are described in Table 1 (Agriculture and Environment Research Unit, 2014). SDS (purity >99%) was supplied by Merck (Germany). Methanol, 1-butanol and 1-pentanol (HPLC grade) were from Scharlab (Spain). Sodium dihydrogen phosphate monohydrate, hydrochloric acid, sodium hydroxide and 1-propanol were ordered from Panreac (Spain). Ultrapure water was in-situ generated using a Simplicity UV ultrapure water generator device (Millipore S.A.S., France). This ultrapure water was used to prepare all the aqueous solution throughout the whole work.

1.2. Solutions and mobile phase preparation

Stock solutions containing 100 µg/mL of each pesticide were prepared by weighting the appropriate amount of solid standard and solving in methanol, and stored at -4°C. Standard solutions were prepared by successive dilutions of

Table 1 – Structure, pK_a and $\log P_{o/w}$ for the studied pesticides (Agriculture and Environment Research Unit, 2014).

Compound (group)	Structure	pK_a	$\log P_{o/w}$
Thiabendazole (benzimidazole)		4.73/12.00	2.39
Pyrimethanil (anilinopyrimidine)		3.52	2.84
o-Phenylphenol (phenol)		9.4	3.18
Imazalil (imidazole)		6.49	2.65

stock solutions in water or in wastewater from sample 12, free of the studied fungicides. These solutions were not stored.

The mobile phases were prepared by weighting the adequate amount of SDS and sodium dihydrogen phosphate. These reagents were solved in water and the pH was adjusted by adding 0.1 mol/L HCl or 0.1 mol/L NaOH. The adequate volume of alcohol was added to achieve the desired concentration. The solution was filled up with water to reach the final volume, ultrasonicated and filtered through 0.45 μm nylon membranes (Micron Separations, USA).

1.3. Apparatus and instrumentation

Solids were weighted using a Mettler–Toledo analytical balance (Switzerland). The pH measurements were performed using a GLP 22 potentiometer (Crison, Spain) equipped with a combined Ag/AgCl/glass electrode. An Ultrasons-H ultrasonic bath (Selecta, Spain) was used to achieve the complete dissolution of the mobile phases.

The chromatographic system was an Agilent Technologies Series 1100 (USA). It was equipped with an isocratic pump, a degasser, an autosampler and UV–Visible diode array detector (DAD). The signal was acquired by a personal computer connected to the chromatograph by means of an Agilent Chemstation version B.01.01.

Chromatograms were treated using Michrom software (Torres-Lapasió, 2000) to extract the chromatographic parameters: retention time (t_R), retention factor (k_R), dead time (t_0), efficiency (N), asymmetry (B/A) and peak area (A). The meaning of the chromatographic parameters has been described by the CHROMacademy (2015).

1.4. Chromatographic conditions

The stationary phase chosen for the analysis was coated in a Kromasil C18 column, with the following characteristics: length

150 mm; internal diameter, 4.6 mm; particle size, 5 μm ; pore size 100 Å. The selected mobile phase was an aqueous solution of 0.07 mol/L SDS — 5%, V/V 1-pentanol — 0.01 mol/L phosphate buffer at pH 3, flowing at 1 mL/min in isocratic mode at room temperature. The injection volume was 20 μL . The detection was performed by switching the absorbance wavelength as follows: 0–9.20, 305 nm; 9.20–12.0, 265 nm; 12.0–20.0, 220 nm. The special care required for liquid chromatographic instrumentation when dealing with micellar mobile phases has been detailed by Rambla-Alegre et al. (2010).

1.5. Sample preparation

Wastewater samples were collected and supplied to the laboratory by Fomento Agrícola Castellonense, S.A. (FACSA, Spain), a company managing the integral water cycle and the evaluation of the water quality in the Spanish province of Castellón. The samples were taken from fruit-processing wastewater, in the influent and effluent stream water in wastewater treatment plants, as well as in the agricultural sewage water (Table 2). The samples were kept in laboratory at 4°C and analyzed in less than three days. Before the analysis, the samples were put out the fridge and maintained in the laboratory for 30 min to warm up to room temperature.

The standard solutions and water samples were analyzed by filtering an aliquot using a 0.45 μm nylon membrane and directly injected in the chromatographic system.

2. Results and discussion

2.1. Optimization of chromatographic conditions

The stationary phase, flow-rate and injection values were taken as the standard values used in MLC, a C18 column, 1 mL/min and 20 μL , respectively. The composition of the

Table 2 – Concentrations ($\mu\text{g/mL}$) of TBZ, PYR, OPP and IMZ detected in sewage water samples.

Origin of water sample	Sample	Location	TBZ	PYR	OPP	IMZ
Sewage agricultural water	1	Villareal	n.d.	0.25–0.5	n.d.	n.d.
	2	La Vilavella	0.18–0.5	0.6	n.d.	n.d.
	3	Betxí	n.d.	0.25–0.5	n.d.	n.d.
	4	Alcora	0.18–0.5	n.d.	n.d.	n.d.
	5	Onda	0.18–0.5	0.7	n.d.	n.d.
	6	Nules	n.d.	1.0	n.d.	n.d.
Collector basin of wastewater from a citrus-processing plant	7	Real Export (Vila-real)	2.0	1.0	n.d.	1.4–4
	8	Invicto (Vila-real)	1.4	n.d.	1–3	n.d.
	9	Serifruit (Vila-real)	0.9	1.5	1–3	n.d.
	10	Eurococi (Betxí)	1.7	1.3	n.d.	1.4–4
Wastewater from WWTP	11	Influent (Nules-La Vilavella)	1.3	0.8	n.d.	n.d.
	12	Effluent (Nules-La Vilavella)	n.d.	n.d.	n.d.	n.d.
	13	Influent (Vora Riu)	1.4	1.1	1–3	1.4–4
	14	Effluent (Vora Riu)	n.d.	n.d.	n.d.	n.d.
	15	Influent (Mancomunada OBVA)	0.6	n.d.	3.1	n.d.
	16	Effluent (Mancomunada OBVA)	n.d.	n.d.	n.d.	n.d.

n.d. not detected.
WWTP: wastewater treatment plants.

mobile phase (SDS, alcohol and pH) and the detection wavelength were optimized. The standard solution used for the optimization analysis was a mixture of 2 $\mu\text{g/mL}$ of TBZ and PYR, 5 $\mu\text{g/mL}$ of OPP and 7 $\mu\text{g/mL}$ of IMZ solved in ultrapure water.

2.1.1. pH selection

The pH values can modify the retention mechanism of the pesticides with acidic/basic activity. The mobile phase was buffered to avoid the oscillations of pH can affect the retention conditions. As the chosen column has a working pH range of 1.5–7.5, only neutral and acidic pHs were tested.

The influence of the pH was evaluated by analyzing the standard solution of the pesticides using the optimal mobile phase buffered at pH 3; 5 and 7. For the four pesticides, the retention times were similar at the three studied pH. However, a strong tailing was observed for TBZ at pH 5 and 7; but at pH 3 the obtained peak was quite Gaussian. It was observed that the shape of PYR, OPP and IMZ do not change by varying the acidity of the mobile phase. Therefore, the pH 3 was selected as optimal.

2.1.2. Selection of surfactant and alcohol

The selected surfactant was SDS, the most widely used in MLC, according to its moderate CMC (8.3 mmol/L), low Krafft point (15°C), high solubility in water, biodegradability and low viscosity of the solutions (Peris-Vicente et al., 2014).

According to the medium-high $\log P_{o/w}$, the pesticides are too hydrophobic to be eluted at a reasonable retention time using a C18 column and a pure aqueous SDS solution them in a useful retention time using a C18 column. The addition of a short chain alcohol would be necessary to increase the elution power of the mobile phase to obtain more adequate retention times, and additionally improve the efficiency (Berthod and García-Álvarez-Coque, 2000). Therefore, the selection of the alcohol was performed by studying the retention times obtained by analyzing the standard solution at several hybrid mobile phases containing 1-propanol, 1-butanol and 1-pentanol.

The concentrations of SDS and organic modifier in the tested mobile phases were chosen following a full factorial design plus the center: four points, combining the minimum and maximum concentration of SDS and alcohol typically recommended for MLC, and a central point taking the intermediate concentrations (Peris-Vicente et al., 2014). In this case, the studied mobile phases were aqueous solutions buffered at pH 3 of:

SDS/1-propanol (mol/L/%, V/V): 0.05/2.5; 0.15/2.5; 0.10/7.5; 0.15/2.5 and 0.15/12.5.

SDS/1-butanol (mol/L/%, V/V): 0.05/1; 0.15/7; 0.10/4; 0.15/1 and 0.15/7.

SDS/1-pentanol (mol/L/%, V/V): 0.05/2; 0.05/6; 0.10/4; 0.15/2 and 0.15/6.

The four pesticides show a binding behavior with SDS-micelles: their retention time decreases at higher concentrations of SDS. Moreover, the retention time also diminishes by increasing the concentration and the length of the carbon chain, as it is usual in MLC. In nearly all the tested mobile phases, the elution order was maintained: t_R (IMZ) > t_R (OPP) > t_R (PYR) > t_R (TBZ). Besides, even using the mobile phase with the higher elution power (0.15 mol/L/6% 1-pentanol), the less retained pesticide (TBZ) was eluted enough far (≈ 4 min) from the dead time (1.04 min).

The use of 1-propanol was discarded, because even at the more eluent conditions (0.15 mol/L/12.5%, V/V 1-propanol), the retention time of IMZ was too high. Comparing 1-butanol and 1-pentanol, this last one provide less retention times in all the SDS/alcohol amount combinations. Therefore, 1-pentanol was selected as the more adequate organic modifier.

2.1.3. Optimization of SDS/1-pentanol concentration

The optimization criterion was to obtain a mobile phase that allows the complete separation of the pesticides in an appropriate analysis time. The concentration of SDS and 1-pentanol were simultaneously optimized using an

interpretative strategy based on a statistical model described by Esteve-Romero et al. (2005). It allows the prediction of the chromatographic behavior of each analyte depending on the composition of the mobile phase.

The retention factor of a compound is related to the concentration of SDS and 1-pentanol in the mobile phase using the following equation:

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

where, $[M]$ (mol/L) and φ (% V/V) are the concentration of SDS and 1-pentanol. The constants mean partition coefficients characteristics of each analyte, and has been described by Esteve-Romero et al. (2005). Besides, the peak shape, and then the efficiency and asymmetry, were modeled using the Eq. (2), which calculates the signal $h(t)$ at each time of the chromatographic run (t):

$$h(t) = H_0 e^{-0.5 \left(\frac{t-t_R}{s_0 + s_1(t-t_R)} \right)^2} \quad (2)$$

where, s_i are constants depending on t_R (min), N (number of theoretical plates) and B/A (dimensionless) (Esteve-Romero et al., 2005). They are ideally the same for each studied compound and mobile phase. H_0 (absorbance unit) depends on the concentration of the pesticide.

Michrom software (Torres-Lapasió, 2000) requires the experimental values of retention factor, N and B/A obtained at five mobile phases to adjust the Eqs. (1) and (2). Thus, the values for each pesticide obtained using the SDS/1-pentanol mobile phases described in Section 2.1.2 were processed. Once known, the statistical model was able to estimate the theoretical values of the chromatographic parameters (retention factor, efficiency and asymmetry) and peak shape for TBZ, PYR, OPP and IMZ at intermediate concentrations of SDS/1-pentanol (0.05–0.15 mol/L/2%–6%, V/V, respectively) by interpolation. It can utilize this information to draw the corresponding simulated chromatograms without performing the analysis and to calculate the theoretical resolution (r_{ij}) for each pair peak (following the valley-peak criterion) and the predicted global resolution of the chromatogram (Z) calculated as the least r_{ij} (Esteve-Romero et al., 2005).

Applying the maximum resolution-minimum analysis time, the optimal mobile phase proposed by the statistical model was 0.07 mol/L SDS — 5%, V/V 1-pentanol at pH 3 ($Z = 0.9997$). The pesticide standard solution was analyzed ($n = 3$) under these conditions (Fig. 1). The obtained chromatogram show completely resolved peaks with adequate shape in <18 min. This indicates the specificity of the method, because each pesticide can be unambiguously identified. The experimental chromatographic parameters (t_R ; N ; B/A) were: TBZ (8.29; 2272; 1.098), PYR (10.95; 3398; 0.973), OPP (13.19; 2176; 1.159), IMZ (15.70; 6637; 12.78). The error in the prediction of the retention factor was <5%.

The selected mobile phase permits the resolution of the pesticide mixture in a short time. Besides, as the isocratic run is used, the stabilization time between two injections is not needed. For these reasons, the achievement of many successive

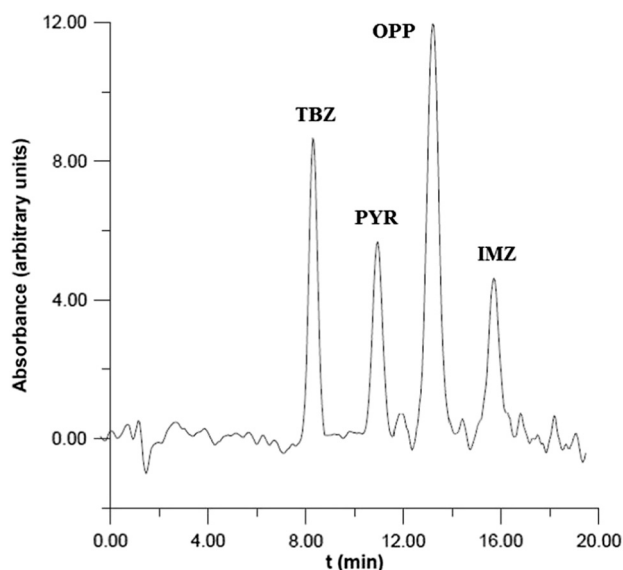


Fig. 1 – Chromatogram obtained by the analysis of a standard solution of 2 μ g/mL of thiabendazole (TBZ) and pyrimethanil (PYR), 5 μ g/mL of *o*-phenylphenol (OPP) and 7 μ g/mL of imazalil (IMZ) in water.

chromatographic runs is facilitated. Moreover, the mobile phase contains lower amount of toxic solvent (5%) than hydroorganic HPLC (up to 100%), and only requires inexpensive reagents and basic chromatographic instrumentation.

2.1.4. Detection conditions

The pesticide standard solution was analyzed using the optimal micellar mobile, and UV-Visible spectra of each pesticide were registered between 200 and 400 nm using a diode array detector. Therefore, the spectra of each pesticide were obtained for each pesticide in the same environment furthermore used for the analysis.

The maximum absorbance wavelength of each pesticide was taken as the optimal value for the analysis: 305 nm for TBZ, 265 nm for OPP and 220 nm for PYR and IMZ. The baseline noise is higher for PYR and IMZ, because 220 nm is a less selective wavelength, and the absorption of the background increases, especially for aqueous solutions. The wavelength detection was then modified during the chromatographic run to quantify each pesticide at its optimal value.

2.2. Method validation

The method was in-house validated following the US EPA review for chemical methods of analysis, applicable for the analysis of environmental water samples, to check the concentration range of applicability and the reliability of the obtained data. The studied validation parameters were: linearity, quantitation range, inter- and intraday trueness and precision, and ruggedness (The FEM Method Validation Team, 2005). The whole validation was performed using standard solutions of TBZ, OPP, PYR and IMZ in effluent wastewater from a WWTP (sample 12), initially without the analytes. The four pesticides were simultaneously studied.

Table 3 – Calibration parameters and quantitation range (concentrations in µg/mL).

Fungicide	Slope	y-Intercept	r ²	LOD	LOQ
TBZ	110.3 ± 1.0	0 ± 14	0.99990	0.18	0.5
PYR	81.2 ± 1.5	5 ± 6	0.99990	0.25	0.5
OPP	89.3 ± 1.3	-50 ± 30	0.9992	1.0	3.0
IMA	38.5 ± 1.1	-48 ± 6	0.9990	1.4	4.0

N = 5; LOD: limit of detection; LOQ: limit of quantification.

2.2.1. Quantitation range and linearity

For calibration studies, several solutions containing increasing concentrations of pesticides were analyzed (n = 3) at increasing concentrations up to 15 µg/mL. The lowest concentration was different for each analyte (see Table 3). The slopes, y-intercepts and determination coefficients were obtained by plotting the peak area versus the corresponding concentration by the least-square linear regression. This procedure was carried out five days during a 3-months period, and the calibration curve parameters were taken as the average values of the five measurements. The quantization range covers from the limit of quantification (see below) to 15 µg/mL.

The limit of quantification (LOQ) is the minimal concentration at which the analyte can be reliably quantified. It was taken as ten times (10 s-criterion) the standard deviation of the blank, taken as the standard deviation of the residual, divided by the sensitivity (slope of the calibration curve) (Esteve-Romero et al., 2005). Levels under LOQ were removed, and the calibration parameters changed accordingly. The final results are shown in Table 3. The excellent values for determination coefficient (r² > 0.9990) indicate a good linearity in the considered range.

The limit of detection (LOD) is the lowest concentration that provides a signal significantly over the baseline noise. Between LOD and LOQ, the analyte is detected, but it cannot be quantified with enough trueness and precision. The LOD was determined as 3 times the standard deviation of the blank divided by the sensitivity (3 s-criterion) (Miller and Miller, 2010).

2.2.2. Trueness and precision

For the intraday measurements, the standard solution was analyzed 6-times in the same day. Intraday precision (repeatability) was determined as the relative standard deviation (RSD) of the peak areas. The intraday trueness was calculated as the quotient of the average value of the concentration provided by the method and true value. For the interday values, the same procedure was performed five different days over a 3-months period, by renewing the solution at each occasion. The interday precision (reproducibility) was the RSD of all the taken peak area, whereas the interday trueness was calculated as the average value of five intraday values.

The results are shown in Table 4. The measures show good recovery (93.9%–103.7%) and low variability (<9.2%) for the quantitative data provided by the method. The high quality of the results is due to the quantitative transferring of the aliquot by the direct injection, which reduces the probability of loss of analyte.

2.2.3. Ruggedness

The modification of the elution power and the sensitivity at slight, but deliberate changes in the main chromatographic parameters (SDS concentration, 1-pentanol, pH and flow-rate) are studied. The influence of each parameter was separately studied by analyzing (n = 3) the standard solution using three mobile phases at: the optimal value, slightly under and slightly over, and remaining the others constant. The RSD of the three measurements was calculated for retention time and peak area of the three measurements. The results are shown in Table 5.

The method was considered quite robust, as low variations were observed for retention times (<9.9%) and peak area (<8.8%).

2.3. Stability in surface water

The stability of the pesticides in water under environmental conditions was evaluated. Thus, the effect of the oscillations of the temperature and the irradiation was considered. A solution of 10 µg/mL of TBZ and PYR, and 15 µg/mL of OPP and IMZ spiked in water was kept for eight days reproducing the

Table 4 – Intra- and inter-day trueness and precision for the studied pesticides.

Pesticide	Concentration (µg/mL)	Intra-day ^a		Inter-day ^b	
		Trueness (%)	Repeatability (RSD, %)	Trueness (%)	Reproducibility (RSD, %)
TBZ	0.5	102.9	4.1	93.9	8.1
	2	101.1	3.2	97.2	1.3
	15	98.7	6.3	98.3	2.9
PYR	0.5	101.8	6.9	103.7	2.8
	2	98.6	4.4	96.5	1.9
	15	97.8	2.9	98.5	5.4
OPP	3	103.2	3.6	95.5	8.3
	5	103.0	5.1	101.2	3.5
	15	98.9	4.5	103.2	4.7
IMZ	4	94.5	9.2	98.7	7.2
	7	104.3	2.1	103.3	0.6
	15	97.8	4.4	97.8	3.8

^a n=6.

^b n=5.

Table 5 – Evaluation of the ruggedness of the MLC-method.

Pesticide	Chromatographic parameters	Level	Retention time (RSD, %)	Peak area (RSD, %)
Thiabendazole	SDS concentration (mmol/L)	65–75	4.3	2.6
	1-Pentanol amount (% V/V)	4.9–5.1	4.0	0.8
	pH	2.9–3.1	3.5	2.5
	Flow rate (mL/min)	0.95–1.05	5.4	3.1
Pyrimethanil	SDS concentration (mmol/L)	65–75	5.0	4.8
	1-Pentanol amount (% V/V)	4.9–5.1	3.1	4.5
	pH	2.9–3.1	5.5	1.1
	Flow rate (mL/min)	0.95–1.05	5.6	3.3
Phenylphenol	SDS concentration (mmol/L)	65–75	4.8	8.6
	1-Pentanol amount (% V/V)	4.9–5.1	4.5	2.3
	pH	2.9–3.1	3.1	3.6
	Flow rate (mL/min)	0.95–1.05	5.4	8.8
Imazalil	SDS concentration (mol/L)	65–75	2.9	1.6
	1-Pentanol amount (% V/V)	4.9–5.1	9.9	5.5
	pH	2.9–3.1	6.5	4.5
	Flow rate (mL/min)	0.95–1.05	5.9	8.2

n=3.

outdoor weather conditions: without controlling the temperature, under the sunlight and warm during the day and in darkness and cold during the night. The flask was thoroughly sealed to avoid water evaporation. An aliquot was analyzed each day (nearly at noontime), and the peak area was measured for each pesticide. The results can be seen in Fig. 2.

The four pesticides show a detectable and continuous degradation process. On the first day, the peak area has diminished (5%–10%). This decrease continues during the following days, and the analytes become undetectable at the eighth day. TBZ undergo a slow decomposition rate during the first six days, since the peak area diminishes nearly 20% during this period. Furthermore, the degradation accelerated, and in only two days the concentration of TBZ falls up to below the detection limit. PYR and OPP show a similar behavior, the peak area lessens at a nearly constant rate of 7% per day during the seven first days, and fully decompose in one more day. On the other hand, IMZ undergoes a rather linear degradation during seven days, when the concentration attains undetectable values.

It was deduced that the four pesticides are significantly affected by the sun radiation and the high temperatures in aqueous media, and remain a short period. Even using high concentration as initial conditions, they fall to undetectable levels in only eight days.

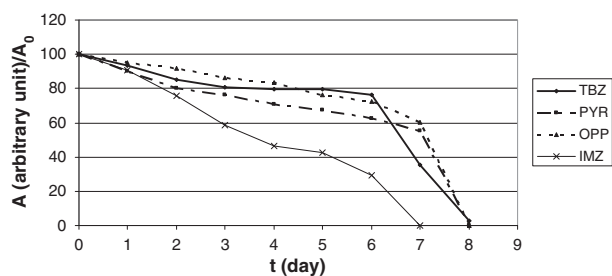


Fig. 2 – Plot of the ratio peak area/initial peak area for the studied pesticides v.s. storage time under outdoor conditions.

2.4. Analysis of wastewater samples

The concentration of TBZ, PYR, OPP and IMZ was determined in several samples from agricultural sewage, fruit-processing industry waste, and WWTP influent and effluent water from the Castelló area. The origin and the concentrations can be seen in Table 2. The analytes were resolved without interferences. Fig. 3 shows the chromatogram obtained by analysis of sample 15.

2.4.1. Optimization of the experimental procedure

Several samples had sludge or oily drops suspended in the aqueous matrix. These samples were filtered, and directly injected, as the aliquot. The pressure does not change, and then neither precipitation nor obstruction in the needle, column or chromatographic tubes was noticed. Thus, the sample was not diluted, allowing to keep a reasonable sensitivity level.

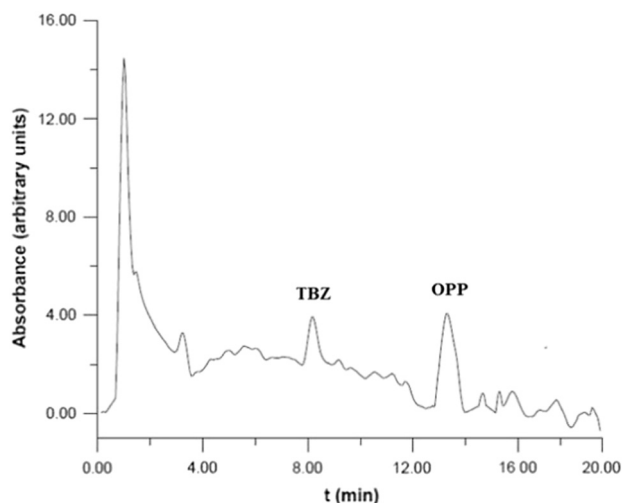


Fig. 3 – Chromatogram of sample 15, collected from the influent water stream of a wastewater treatment plant.

This possibility of direct injection, previous filtration, without dilution, clean-up or extraction step can be considered as the main advantage of the method. The simplification of the experimental procedure allows to reach good quality of the validation results, while minimizing the participation of trained staff, and strongly shortening the analysis time, then favoring the analysis of a large amount of samples. Besides, neither chemical nor extraction instrumentation are needed, reducing the cost, the environmental impact and the safety of the operator.

2.4.2. Results in real samples

Wastewater samples from agricultural origin show a significant concentration of TBZ and PYR. This indicates that these pesticides are pre-harvest applied to crops, and remain in sludge, prior to be dragged to rain water to the wastewater. The collector basins of the fruit-processing plants contain a significant amount of TBZ, OPP and IMZ, those used for post-harvest protection. However, considering the large quantity of pesticides needed to assess a correct storage of the fruits, the results indicate that the plants have implemented a purification treatment to partially purify the wastewater prior discharge.

The influent stream of WWTPs contains a moderate concentration of the TBZ, PYR, OPP and in one case, IMZ, because they come from areas with a strong agriculture-related activity. The pesticides in effluents were lower than in influent, ensuring the validity of the water purification process.

3. Conclusions

The here-described MLC-method can be used to monitor TBZ, PYR, OPP and IMZ in routine analysis of sewage water. The sample was directly injected into the chromatographic system after a simple filtration, thus avoiding complex and time consuming intermediate steps. The analytes were identified and eluted without interferences from the wastewater matrix in less than 18 min. As main features, we highlight the low global analysis time, and the easy-to-handle sample preparation, which permits the analysis of a large amount of samples per day. The method was validated in terms of quantitation range, linearity, precision, trueness and ruggedness, following the Validation and Peer Review of US EPA for chemical methods of analysis. The method meets the requirements of “green chemistry”, as low amount of toxic reagents are used, then reducing the waste of pollutants and minimizing the danger for the operator health. Besides, the method is quite inexpensive, and then making it accessible even to laboratories with low economic power. The stability of the pesticides in water at outdoor conditions was evaluated. Finally, the method was applied to determine the concentration of TBZ, PYR, OPP and IMZ in sewage water from an area with a strong fruit-related activity, and suspected to be contaminated.

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