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Development and validation of a simple thin-layer chromatographic method for the analysis of *p*-chlorophenol in treated wastewater

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Abstract: A thin-layer chromatographic (TLC) method with densitometric detection was established for the quantification of *p*-chlorophenol in wastewater. Degradation efficiency of *p*-chlorophenol was monitored after each treatment of the wastewater samples. Degradation of *p*-chlorophenol was performed by advanced oxidation processes (AOPs), using UV, H_2O_2/UV, O_3/H_2O_2/UV, O_3 and O_3/UV. The developed TLC procedure was found to be simple, rapid and precise. The method is characterized by high sensitivity (the limit of detection was 11 ng per band and limit of quantification 35 ng per band), a linear range from 75 to 500 ng per band, r = 0.9965), and high precision, accuracy (mean percentage recovery 98.6 %), and specificity. Additionally, the efficiency of degradation was monitored using HPLC giving comparable results with the reversed phase TLC measurements.

Keywords: p-chlorophenol; TLC-scanner; HPLC; AOPs; wastewater treatment.

INTRODUCTION

Chlorophenols are considered as one of the major aquatic pollutants with toxicity for humans and animals. Various chlorophenols are intermediates in synthesis of many pesticides and dyes. In the chlorination process of water, they arise as products of phenol chlorination. As they subsequently enter the aquatic environment, chlorophenols are to be found in surface water, groundwater and especially in wastewater.[1]

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Some of the chlorophenols are slightly biodegradable, while others are more persistent and mobile in the aquatic environment. Their bioaccumulation increases with the introduction of substituents into the phenol ring. O*rtho*-substituted chlorophenols are less toxic than *meta*- and *para*-substituted derivatives.[2]

Wastewater treatment is very important to minimize water pollution and prevent waterborne diseases. The search for ways to treat wastewater in a safe manner with low running costs is constant. Many processes, chemical, physical, biological and a combination of them could be used for water treatment.[3] Standard techniques for water treatment, such as coagulation, carbon adsorption, reverse osmosis, and ultrafiltration, are not efficient in the removal of chlorophenols. One of alternatives for the water treatment is processes based on the formation free hydroxy radicals with very strong oxidation potential, known as advanced oxidation processes (AOPs).[4] These processes lead to complete degradation of chlorophenols to carbon(IV) oxide or to biodegradable and less toxic intermediates.

Previously, the experimental results of the efficiency of *p*-chlorophenol degradation using a falling film dielectric barrier discharge (DBD) reactor were published.[5] Different conditions for the degradation of *p*-chlorophenol in aqueous solution were examined. The kinetics of the degradation in several successive passes through the reactor was monitored using high performance liquid chromatography (HPLC).[5]

The efficiency of degradation processes is usually monitored using techniques such as HPLC and gas chromatography (GC). Literature references indicate that these techniques are the most reliable and the most sensitive methods for such evaluations.[6,7] However, some results could be found for phenol and its derivatives on bonded amino, cyano and diol thin-layer stationary phases.[8] Modern thin-layer chromatography (TLC) is an instrumental technique that is comparable by its accuracy and precision with both HPLC and GC. With high efficiency and significant reproducibility, it became applicable for environmental monitoring.[9,10]

In comparison with HPLC, TLC has several advantages: it is a simple, fast technique, requires little or no sample preparation and clean-up, it is easy to perform, saving both time and expense. The major advantage of TLC is that several samples can be run simultaneously using a small quantity of mobile phase, unlike in HPLC, thus reducing analysis time and cost per analysis. Solvent consumption and the amount of waste associated with the sample preparation in TLC are minimal. This is particularly important from the green chemistry point view.[11] Furthermore, even cruder samples can be analyzed by TLC because each plate is used only once, and therefore development is not as critical as in HPLC, where the possibility of destroying the column occurs.

To this end, a TLC procedure for the determination of *p*-chlorophenol in wastewater samples is presented herein. Main goals were to: *i*) use a simple and

cheap technique for monitoring the removal of *p*-chlorophenol from wastewater samples, *ii*) obtain optimal thin-layer efficiency and *iii*) to validate in such way the adopted thin-layer chromatographic method.

EXPERIMENTAL

Reagents and chemicals

All reagents used were of analytical grade purity. *p*-Chlorophenol (*p.a.*) was purchased from Aldrich (USA). Acetonitrile and triethylamine used for the experiments were purchased from Merck (Germany). Ultrapure water was obtained from a Micropure TKA system (Germany) and used for the preparation of the mobile phases for reversed phase (RP) TLC and HPLC. Wastewater samples and a standard stock solution were prepared by dissolving *p*-chlorophenol in ultrapure water to give a concentration 100.0 mg L^{-1}.

Advanced oxidation processes

The starting concentration of *p*-chlorophenol was 100.0 mg L^{-1}. Five different sets of conditions were examined: UV, H_2O_2/UV, O_3/H_2O_2/UV, O_3 and O_3/UV. A UV flow lamp (BULEGO SH-500, Italy) with a maximum at 253.7 nm and 38 W power was used. The UV lamp was included in all systems, except the O_3 system. The flow rate of the solution (210 mL min^{-1}) was set by a peristaltic pump.

The working principle of an ozonizator is based on an electrical discharge through air, whereby the oxygen is converted to ozone. A Lifepool 1.0 ozonizator (Lifetech, (Czech Republic), was used. The flow rate of the air through the ozonizator, measured by rotameter, was adjusted to 10 L min^{-1}. This model generates 1 g h^{-1} of ozone. A schematic representation of the experimental setup is shown in Fig. 1. In all experiments, the reactor was filled with 750 mL of an aqueous solution of *p*-chlorophenol. Ozone was introduced into the bottom of the reactor (round bottom flask, 1 L) through a glass tube (with sintered-glass diffusers at the end) for 30 minutes at a flow rate 2 L min^{-1}.

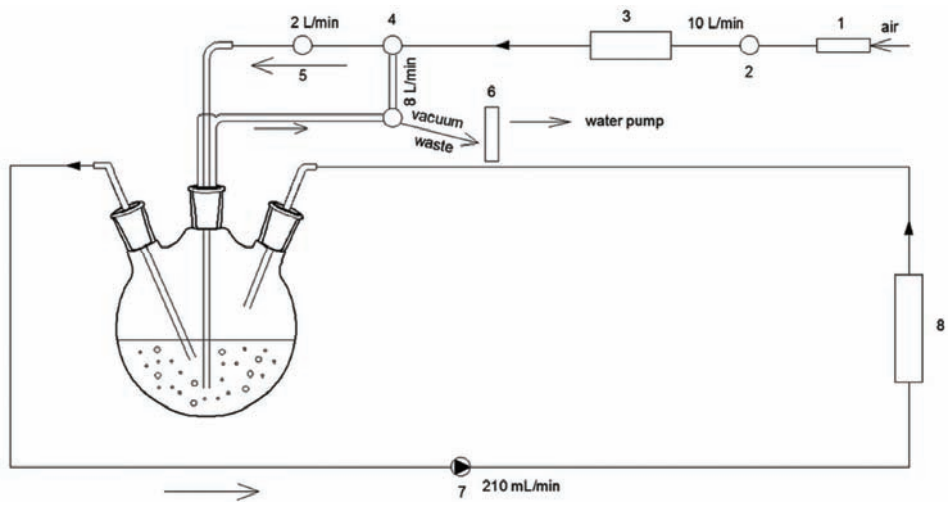

Fig. 1. Schematic diagram of the experimental setup: 1) air drying column, 2) rotameter, 3) ozonizator, 4) three-way valves, 5) rotameter, 6) safety glass vessel, 7) peristaltic pump, and 8) UV lamp.

In the experiments where H_2O_2 was used, the concentration of H_2O_2 was adjusted to 20 mmol L^{-1} and the pH value was set to 9. The removal efficiency was monitored after 5, 10, 20, and 30 minutes from the start of the treatment.

Optimization of the TLC method

To obtain optimal thin-layer efficiency, several parameters were evaluated, including the choice of mobile phase and plate type. Commercially available precoated C 18 plates were chosen as the stationary phase. The applicability of C 18 plates was already suggested in a previous paper when the possibility of separating different mono- and poly-substituted phenols under reversed phase chromatographic conditions was investigated.[10] The reversed phase proved to be good choice for the determination of the partition coefficients of phenols.[10] High performance thin-layer plates (HPTLC C 18 plates) were also investigated, but *p*-chlorophenol eluted with the solvent front and the peak shape was poor. The desired symmetrical and reproducible peak shape was achieved using conventional reversed-phase C 18 plates.

The selection of a suitable mobile phase involved several trials. Different concentrations of standard solution (15–100 ppm) were spotted onto C 18 TLC plates and run in different solvent systems. Different organic modifiers were used in different ratios with water as the mobile phase. Initially, acetonitrile and methanol in different ratios as mobile phase modifiers were selected. Then, mobile phase composition was modified and acetone as solvent modifier gave the optimal chromatogram and a suitable retardation factor, R_F, value. Triethylamine (TEA) and silanol blocking agents were tested as mobile phase additives to reduce zone tailing and improve peak shape, as it was already emphasized in a previous paper.[12] Finally, the optimum mobile phase consisting of acetone:water:TEA in the ratio 60:38:2 (v/v/v) was chosen.

RP TLC densitometric analysis

A standard stock solution containing 100.0 mg L^{-1} of *p*-chlorophenol was freshly prepared in ultrapure water. Five standard solutions (15–100 ppm) were prepared by serial dilution of the standard stock solution. To prepare a calibration curve, aliquots of 5 µl of the standard solutions were applied on the C 18 TLC plate along with the treated water samples (5 µl). Solutions were spotted as bands with a Camag (Muttenz, Switzerland) microlitre syringe onto a RP 18 TLC plates 10 cm×10 cm (1.05559, Merck (Darmstadt, Germany) using a Camag Linomat V sample applicator. A constant application rate (dosage speed 50 nL s^{-1}) was employed.

Linear ascending development was realized in a Camag twin-trough glass development chamber saturated with the mobile phase. The optimum chamber saturation time for the mobile phase was 15 min at room temperature. The plates were developed up to 8 cm. Subsequent to the development TLC plates were dried at room temperature. The plates were scanned with a Camag TLC Scanner 3 with Wincats integration software. Densitometric scanning was performed in the absorbance mode at 240 nm with slit dimensions of 6.00 × 0.30 mm and a scanning speed of 20 mm s^{-1}. A deuterium lamp was used as the radiation source. From the respective calibration curves obtained by plotting the concentrations of standards against the corresponding peak areas, the amounts of *p*-chlorophenol in the water samples were determined.

HPLC Analysis

The HPLC system consisted of a Waters 1525 Binary HPLC pump and a Waters 2487, Dual λ absorbance detector. System management and data acquisition were accomplished with Empower software. The compounds were separated on a Symmetry® 5 µm C 18, Waters column (150 mm× 4.6 mm, 5 µm particle size). Standard solutions of *p*-chlorophenol (5, 10,

25, 50, and 100 ppm) and treated water samples were injected (10 μL) after equilibration of the column. Samples were filtered through 0.45 μm filter prior to analysis. Chromatography was performed at room temperature and the eluate was monitored at 280 nm. A binary mixture of acetonitrile and water was used as the mobile phase at a flow rate of 1 ml min^{-1}. The initial mobile phase was a mixture of acetonitrile and water 30:70 (v/v); then a linear gradient increasing to 80 % acetonitrile in 20 min was applied. For identification and quantitative determination of the *p*-chlorophenol remaining in the water samples, retention time (t_R) and UV spectra were compared with the retention time and UV spectra of the standard solution (t_R = 7.6 min).

Validation procedure of the TLC Method

Validation of the optimized TLC method was performed with respect to the following parameters.

Linearity and range. From the standard stock solution (100.0 mg L^{-1}) of *p*-chlorophenol, standard solutions were prepared, and 5μL of each solution were spotted on a TLC plate to obtain a final concentration of 75–500 ng per band. Each concentration was applied three times on the TLC plate. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

Precision. The repeatability of the method was assessed by analysis of the 375 ng per band of standard solution of *p*-chlorophenol (n = 6) and is expressed as the relative standard deviation (*RSD*, %) and standard error (*SE*) of the peak areas. The variability of the method was studied by analyzing standard solutions of *p*-chlorophenol (250, 375, and 500 ng per band) three times on the same day (intra-day precision) and for three times on three different days over a period of one week (inter-day precision); results were again expressed as *RSD*, %. The instrument precision was assessed by scanning the same band of *p*-chlorophenol (375 ng) six times and is expressed as *RSD*, % of the peak area.

Limit of detection and limit of quantification. The limit of detection (*LOD*) and limit of quantification (*LOQ*) represent the concentrations of the analyte that would yield signal-to-noise ratios of 3 for *LOD* and 10 for *LOQ*. To determine the *LOD* and *LOQ*, serial dilutions of standard solutions were made from the standard stock solution in the range of 75–500 ng per band.

Specificity. The specificity of the method was determined by analyzing a standard solution of *p*-chlorophenol and wastewater samples. The R_F values and the obtained spectra of samples and standard solution were compared. The peak purity was accessed by comparing the spectra at peak start, peak apex and peak end positions of band.

Accuracy. The accuracy of the method was studied by performing experiments using the standard addition technique. Varying amount of standard was added to previously analyzed samples and accuracy was determined at three different levels (50, 80 and 100 %). Known amounts of *p*-chlorophenol (100, 150 and 200 ng) were added to a treated wastewater sample. The results of the recovery are expressed as %.

Robustness. The robustness of the method was studied by introducing small changes (± 0.2 mL for each component) in mobile phase composition (acetone:water:TEA 60:40:2, 60:36:2, 58:38:2 and 62:38:2, v/v/v). The time from spotting to chromatography and from chromatography to scanning was varied from ±10 min. The robustness of the method was determined at a concentration level of 375 ng per band.

RESULTS AND DISCUSSION

Wastewater samples were successively treated for the degradation study using five different sets of conditions: UV, H_2O_2/UV, O_3/H_2O_2/UV, O_3 and O_3/UV. Samples were tested at certain time intervals (5, 10, 20 and 30 min). As expected, concentration of *p*-chlorophenol decreased as a function of the length of time during which the degradation was performed. Degradation products, *i.e.* organic acids (formic acid, acetic acid, and oxalic acid) and chloride intermediates were monitored and quantified using ion chromatography.

Quantitative analysis of p-*chlorophenol by TLC*

Different concentrations of *p*-chlorophenol were plotted against peak area to obtain a calibration plot. A 5-μl aliquot of the treated water samples were applied along with standard solution of *p*-chlorophenol. A representative 3D chromatogram of the standard (tracks 1–3, 12, and 13) and wastewater samples treated by usage of O_3 (tracks 4–7) and O_3/UV, (tracks 8–11), pH = 9 is shown in Fig. 2. The application position was Y = 10.0 mm; band length = 6.0 mm.

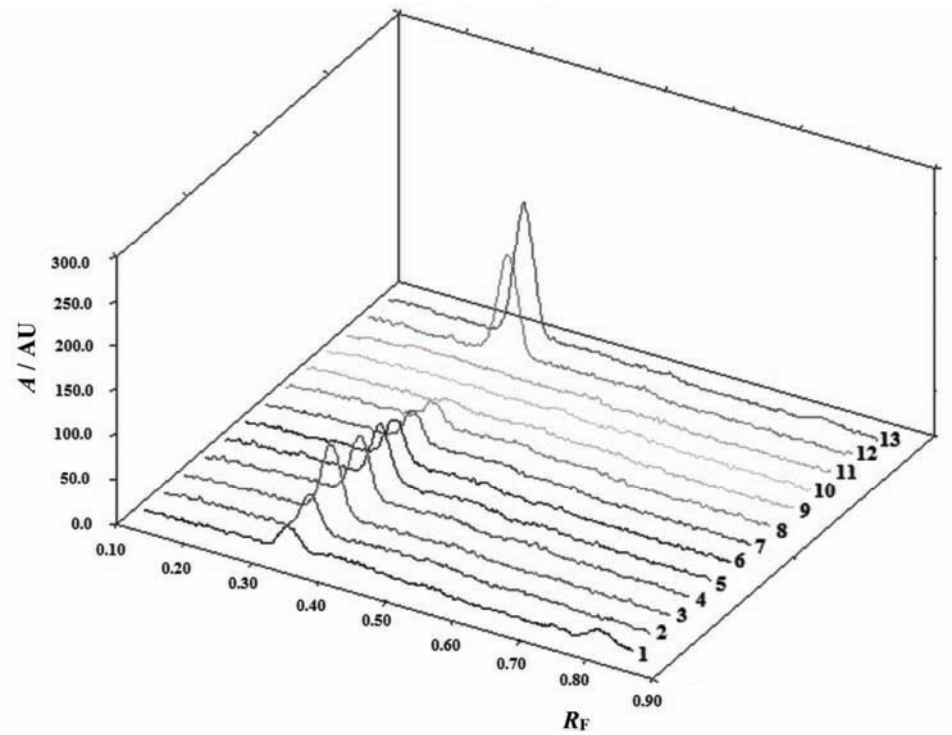

Fig. 2. 3D TLC Densitogram at 240 nm of *p*-chlorophenol standard solutions (tracks 1–3, 12 and 13) and wastewater samples treated with O_3 (tracks 4–7) and O_3/UV (tracks 8–11).

The identity of *p*-chlorophenol in different samples was confirmed by comparing the R_F value and UV spectrum of the peak of the standard with the corresponding peak from the sample. The peaks corresponding to *p*-chlorophenol were on the same R_F value (0.32). The TLC chromatogram of *p*-chlorophenol with the corresponding R_F at 240 nm is depicted in Fig. 3. Comparison of the absorption spectra of *p*-chlorophenol from the standard (250 ng per band) and treated water sample (O_3), measured on the TLC plate (data resolution step = 10 nm) is given in Fig. 4.

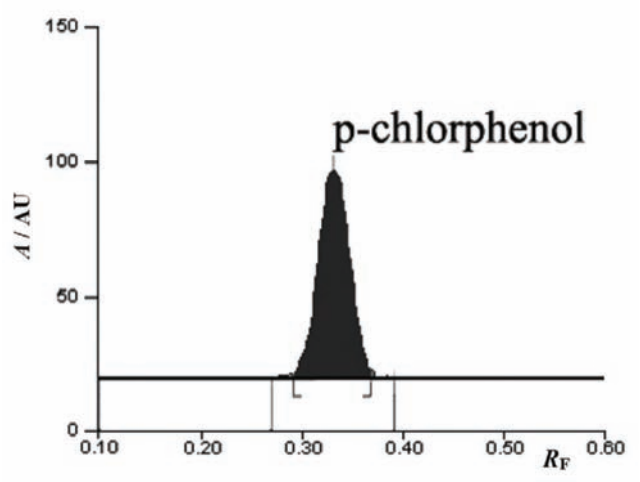

Fig. 3. TLC Densitogram peak display of *p*-chlorophenol standard, 250 ng per band (track 3, R_F 0.32±0.02).

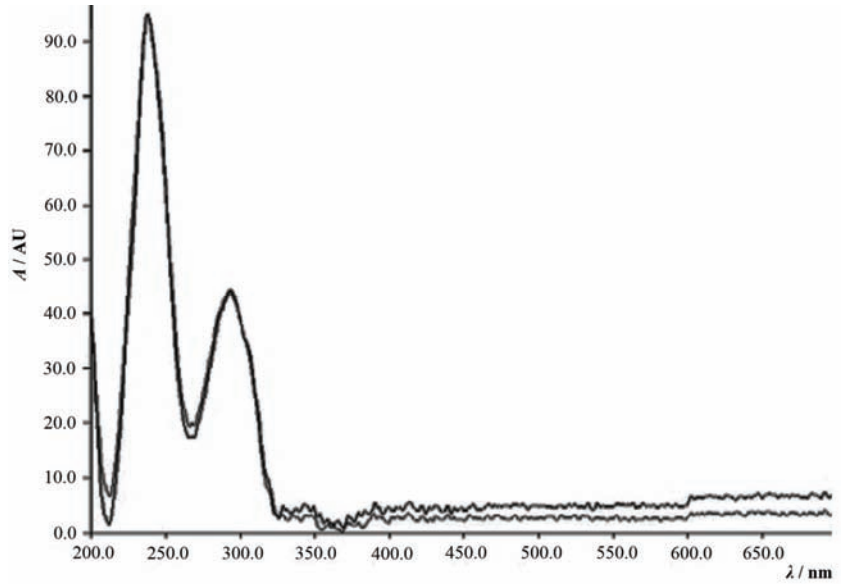

Fig. 4. Overlaid absorption spectrum of *p*-chlorophenol standard and a treated water sample.

Validation of the TLC method

The standard response was linear (r = 0.99986, SD = 1.29) over the concentration range between 75–500 ng per band. The linear regression equation was represented as $Y = 7.63X + 27.39$, where X is concentration of *p*-chlorophenol in ng per band and Y is the peak area.

The results of the repeatability and intermediate precision experiments are given in Table I. The developed method was found to be precise as the *RSD* values for the repeatability and intermediate precision studies were <2 %.

TABLE I. Precision studies for *p*-chlorophenol; instrument precision: 372.2±3.9 (1.0 %)

Taken ng per band	Intra-day (n = 3)		Inter-day (n = 3×3)	
	Measured concentration±*SD* ng	*RSD* / %	Measured concentration±*SD* ng	*RSD* / %
250	252.0±2.1	0.8	251.0±2.9	1.2
375	372.9±2.5	0.7	373.7±2.0	0.5
500	502.0±1.3	0.2	501.2±2.5	0.5

The *LOD* and *LOQ* were calculated from the equations $LOD = 3 \times N/B$ and $LOQ = 10 \times N/B$, where N is the *SD* of the peak area of the standard, taken as a measure of the noise, and B is the slope of the corresponding calibration curve. The *LOD* and *LOQ* were found to be 11 and 35 ng per band, respectively. The method was found to be specific for *p*-chlorophenol.

As shown in Table II, good recoveries of *p*-chlorophenol in the range from 99.2 to 100.5 % were obtained for various added concentrations. The average recovery of three levels (nine determinations) was 98.6 %.

TABLE II. Results of the recovery study (n = 3, ng per band); amount in sample: 182.6 ng per band

Amount in sample	Total amount	Amount detected	Recovery, %
100 (50 % level)	282.6	283.3 ± 1.2	100.2 ± 0.4
150 (80 % level)	332.6	334.4 ± 3.1	100.5 ± 0.9
200 (100 % level)	382.6	379.8 ± 2.2	99.2 ± 0.6

The low values of the *RSD*, given in Table III, indicate the robustness of the method. No significant change in the R_F of *p*-chlorophenol was observed when the composition of the mobile phase was changed slightly. In addition, changing the time interval between chromatography, spotting and scanning had no impact.

TABLE III. Robustness testing (n = 3)

Parameter	*SD* of peak area	*RSD* / %
Mobile phase composition (± 0.2 mL)	2.7	0.7
Time from spotting to chromatography (± 10 min)	3.3	0.9
Time from chromatography to scanning (± 10 min)	3.8	1.0

The standard deviation of the peak areas was calculated for each parameter and the *RSD* was found to be less than 2 %.

Comparison of TLC and HPLC results

The concentration of *p*-chlorophenol remaining in the treated wastewater samples was determined using two different reversed-phase chromatographic methods, TLC densitometry and HPLC with UV detection. The percent degradation of *p*-chlorophenol as a function of the irradiation time, monitored by TLC and HPLC are illustrated in Figs. 5A and 5B, respectively. The results obtained by these two methods were treated as paired data and were compared by the matched pair Student's *t*-test. The calculated *t*-value was 0.40, *i.e.*, below the critical two-tail *t* value of 3.18. Hence, it was concluded that both methods gave comparable results for the analysis of *p*-chlorophenol degradation.

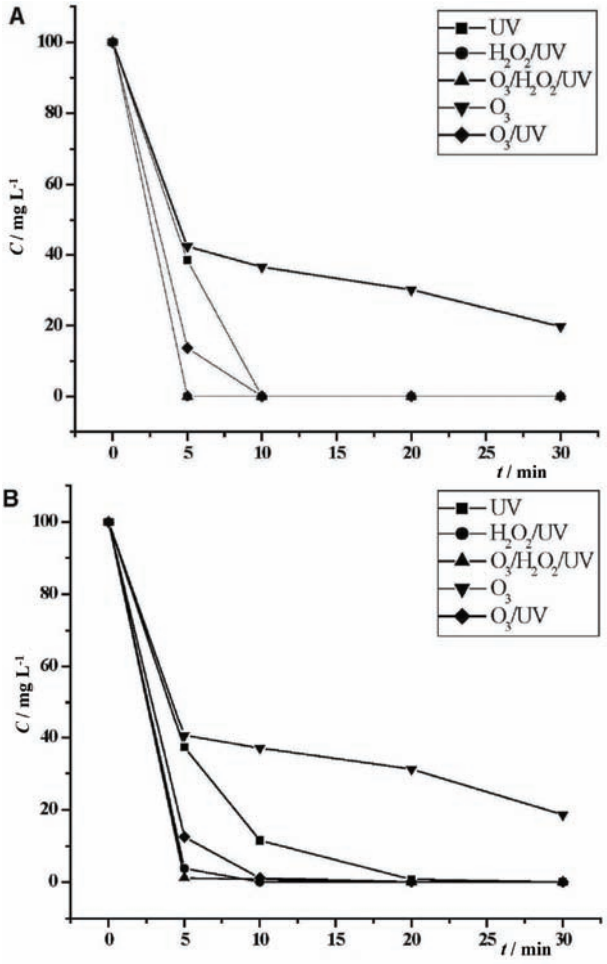

Fig. 5. The change of *p*-chlorophenol concentration as a function of the time using different AOPs, monitored by TLC (A) and HPLC (B).

CONCLUSIONS

The concept of this study was to develop a TLC densitometric method for both qualitative and quantitative determination of *p*-chlorophenol in wastewater samples. The proposed method is cheap and simple, and proved to be suitable for the accurate monitoring of degradation efficiency during the treatment of wastewater samples. The almost identical results obtained using TLC and HPLC, led to the conclusion that both methods could be applied for such investigations. However, the developed TLC method is an attractive alternative to HPLC. The fast analysis time, no sample preparation, low solvent consumption (which in ecological sense makes it more acceptable) and the possibility of analyzing several samples simultaneously, make the TLC the method of choice. In view of the importance of wastewater purification, the proposed method might find many direct applications and could be widely applied for routine analysis of related compounds under environmental control.

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И З В О Д

ВАЛИДАЦИЈА ТАНКОСЛОЈНЕ ХРОМАТОГРАФИЈЕ КАО МЕТОДЕ ЗА ЈЕДНОСТАВНО ОДРЕЂИВАЊЕ *p*-ХЛОРФЕНОЛА У ТРЕТИРАНОЈ ОТПАДНОЈ ВОДИ

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Квантификација *p*-хлорфенола у узорцима отпадних вода урађена је применом танкослојне хроматографије са денситометријском детекцијом. Ефикасност деградације *p*-хлорфенола праћена је након сваког третмана узорка отпадне воде. UV светлост, O_3, као и комбинација H_2O_2/UV, O_3/H_2O_2/UV и O_3/UV су коришћени за деградацију *p*-хлорфенола. Примењен TLC поступак је једноставан, брз и прецизан. Висока осетљивост (лимит детекције је 11 ng по траци и лимит квантификације је 35 ng по траци), опсег линеарности (од 75 до 500 ng по траци, r = 0,9965), висока прецизност и тачност, као и специфичност су карактеристике коришћеног TLC поступка. HPLC метода, као стандардна метода за одређивање *p*-хлорфенола је такође примењена за проучавање ефикасности поступка деградације. Резултати добијени применом TLC методе су упоредиви са HPLC мерењима.

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REFERENCES

1. P. N. Moza, K. Fytianos, V. Samanidou, F. Korte, *Bull. Environ. Contam. Toxicol.* **41** (1988) 678
2. J. Michałowicz, W. Duda, *Pol. J. Environ. Stud.* **16** (2007) 347

3. R. L. Droste, *Theory and practice of water and wastewater treatment*, Wiley, New York, USA, 1997, p. 219
4. W. Z. Tang, *Physicochemical treatment of hazardous wastes*, Lewis Pub., Boca Raton, FL, USA, 2003
5. D. Manojlovic, D. R. Ostojic, B. M. Obradovic, M. M. Kuraica, V. D. Krsmanovic, J. Puric, *Desalination* **213** (2007) 116
6. I. Oller, S. Malato, J. A. Sánchez-Pérez, *Sci. Total Environ.* **409** (2011) 4141
7. C. A. Somensi, E.L. Simionatto, S. L. Bertoli, A. Wisniewski Jr., C. M. Radetski *J. Hazard. Mater.* **175** (2010) 235
8. I. Baranowska, C. Pieszko, *J. Planar Chromatogr.* **11** (1998) 119
9. S. Babić, M. Petrović, M. Kastelan-Macan, *J. Chromatogr. A* **823** (1998) 3
10. F. Lj. Andrić, J. D. Trifković, A. D. Radoičić, S. B. Šegan, Ž. Lj. Tešić, D. M. Milojković-Opsenica, *Chemosphere* **81** (2010) 299
11. C. J. Welch, N. Wu, M. Biba, R. Hartman, T. Brkovic, X. Gong, R. Helmy, W. Schafer, J. Cuff, Z. Pirzada, L. Zhou, *Trends Anal. Chem.* **29** (2010) 667
12. S. Ilić, M. Natić, D. Dabić, D. Milojković-Opsenica, Ž. Tešić, *J. Planar Chromatogr.* **24** (2011) 93.