



Derivative spectrophotometric determination of the herbicides picloram and triclopyr in mixtures

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Abstract: A derivative spectrophotometric method for the determination of the herbicides picloram (4-amino-3,5,6-trichloropicolinic acid) and triclopyr (3,5,6-trichloro-2-pyridyloxyacetic acid) in mixtures was developed in this work. Derivative spectrophotometric determination of the selected herbicides was preceded by investigations concerning the influence of pH, in the pH interval from 1 to 9. At pH 3.2, picloram and triclopyr solutions are stable under daylight for nine months. This pH was used for all subsequent determinations. It was also found that the use of the first derivative of the spectra at 232 nm was optimal for the determination of picloram, while use of the second derivative of the spectra at 211 nm was best suited for the determination of triclopyr. The calibration curves are linear in the concentration range 0.8 – 13 $\mu\text{g cm}^{-3}$ with correlation coefficients –0.9998 for picloram and 0.9996 for triclopyr. The limit of detection of the developed method is 0.08 $\mu\text{g cm}^{-3}$ for picloram and 0.03 $\mu\text{g cm}^{-3}$ for triclopyr. Derivative spectrophotometry was shown to be an appropriate method for the determination of picloram and triclopyr in mixtures and in pesticide formulations, unlike the deconvolution method.

Keywords: picloram, triclopyr, mixture, derivative spectrometry, zero-crossing method, deconvolution.

INTRODUCTION

Picloram (4-amino-3,5,6-trichloropicolinic acid, M_r 241.46, Fig. 1a) and triclopyr (3,5,6-trichloro-2-pyridyloxyacetic acid, M_r 256.47, Fig. 1b) are selective systemic herbicides, in the chemical class of pyridine compounds, used worldwide to control most annual and perennial broad-leaved weeds in lawns, turf, pastures, rights-of-ways and various crops, such as wheat, barley and oats.¹ They are used in the form of salts or esters as active components in different pesticide formulations individually and in mixtures. These formulations, besides the active components, generally, also contain one or more inert ingredients, such as liquid hydrocarbons, ethylene glycol, diethylene glycol monoethyl ether, polyglycol, ethanol etc.²

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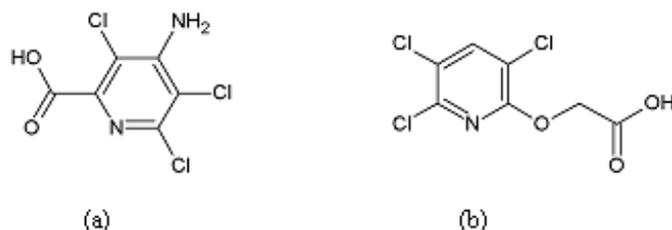


Fig. 1. Structures of (a) picloram and (b) triclopyr.

AOAC for determination of picloram and/or triclopyr provides the only official method for the determination of picloram and 2,4-D (2,4-dichlorophenoxyacetic acid) in mixtures in pesticide formulations, by applying a liquid chromatographic method.³

Spectrophotometric analysis, on the other hand, offers a fast and reliable method for solving different analytical problems. However, in conventional quantitative spectrophotometric methods based on the measurement of the absorbance of the analyte at a given wavelength, it is very common, especially in analysis of complex mixtures, for the analyte to be accompanied by other compounds exhibiting interfering signals. In such cases, the derivative method offers a useful means for improving the resolution of the determination of mixtures, because it enhances the detectability of minor spectral features.⁴⁻⁷ Namely, derivative spectrophotometry is based on use of derivative spectra which are generated from the zero-order spectra. Separation of overlapped signals and elimination of background caused by presence of other compounds in a sample can be achieved by derivatization, enabling the determination of one or more analytes without prior separation or purification.⁴⁻⁷ Derivative spectrophotometry is an analytical technique which has proven to be of great use for obtaining qualitative and quantitative information from spectra composed of unresolved bands by using the first or higher derivatives of the absorbance with respect to wavelength. It emphasizes subtle spectral features by enabling the resolution of multi-component elements and reducing the effect of spectral background interferences. This technique offers an alternative approach to the enhancement of sensitivity and specificity in the analysis of mixtures. The derivative transformation allows discernment against broad band interferences which arise from turbidity or non-specific matrix absorption.

As already stated, picloram and triclopyr are commercially available in pesticide formulations, either individually or in mixtures, as well as with inert ingredients. For this reason, the objective of this work was to develop a derivative spectrophotometric method for their determination in mixtures. The developed method was validated by determination of these herbicides in synthetic mixtures, as well as in formulations. In addition, the aim of this work was to compare the results obtained by derivative spectrophotometry to those obtained by using the deconvolution mode of the software provided with the spectrophotometer.

EXPERIMENTAL

Chemicals and solutions

Picloram (97.4 %) and triclopyr (99.8 %) were both purchased as analytical standards from Riedel–de Haën. Boric acid, *p.a.*, was purchased from Kemika, Zagreb, phosphoric acid, 85 %, *p.a.*, from Lachema, Neratovice, while sodium hydroxide, *p.a.*, was purchased from ZorkaPharm, Šabac. All solutions were made using doubly distilled water.

Stock solutions of the investigated herbicides ($\sim 250 \mu\text{g cm}^{-3}$) were prepared for the investigations. Standard solutions for the construction of the calibration curve ($0.8\text{--}13 \mu\text{g cm}^{-3}$) were prepared by measuring the required volumes of stock solutions into 10 cm^3 volumetric flasks. Then, 2 cm^3 of Britton–Robinson (BR) buffer pH 3.2 were added and the solutions were made to mark with doubly distilled water. For investigations of the influence of pH on the absorbance of picloram and triclopyr, solutions containing $13 \mu\text{g cm}^{-3}$ of each herbicide and 2 cm^3 of BR buffer of the appropriate pH value, in the interval from 1.0 to 9.0, were prepared. In the case of investigation of stability of solutions of picloram and triclopyr, two series of solutions containing $\sim 25 \mu\text{g cm}^{-3}$ of herbicides at pH 3.2 were prepared. One series was left to stand in the light, near a window, while the other was a control series which was for that reason left in the dark, also at room temperature. Spectra were recorded periodically during 9 months.

The three investigated synthetic binary mixtures were obtained by measuring appropriate volumes of stock solutions into 10 cm^3 volumetric flasks to make mixtures in which picloram and triclopyr were in the following concentrations: $4 \mu\text{g cm}^{-3}$ picloram and $12 \mu\text{g cm}^{-3}$ triclopyr (mixture 1), $6 \mu\text{g cm}^{-3}$ picloram and $6 \mu\text{g cm}^{-3}$ triclopyr (mixture 2) and $12 \mu\text{g cm}^{-3}$ picloram and $4 \mu\text{g cm}^{-3}$ triclopyr (mixture 3). These solutions also contained 2 cm^3 of pH 3.2 BR buffer.

Furthermore, the developed method was used for the determination of triclopyr in Garlon 3A, a formulation of this herbicide which, besides triclopyr in form of the triethylamine salt, contained ethanol, triethylamine and ethylenediaminetetraacetic acid as inert ingredients. The content of the acid equivalent of triclopyr was 31.8 %. For this purpose, a stock solution of Garlon 3A, in which the triclopyr concentration was $\sim 250 \mu\text{g cm}^{-3}$, was prepared. This solution was further diluted to prepare solutions with triclopyr concentrations of $\sim 12 \mu\text{g cm}^{-3}$. These determinations were also carried out at pH 3.2 by addition of BR buffer.

Unfortunately, a formulation containing a mixture of picloram and triclopyr is not available for purchase on the Serbian market. For this reason, such a mixture was prepared by adding a solution of picloram to an appropriate aliquot of Garlon 3A in an amount to obtain a mixture which contained triclopyr and picloram in the ratio 3:1; the ratio in which these two herbicides are found in commercially available Grazon[®]DS. In the said formulation, triclopyr is present as the butoxy-ethylester and picloram as the hexyloxypropylamine salt.

Analytical procedure

Spectra of all solutions were recorded in the wavelength region from 200 to 325 nm on an Anthelie version 2 spectrophotometer (Secomam, France), in 1 cm quartz cells with a fixed slit width (1 nm) at scan rate 1200 nm min^{-1} . Triclopyr was determined either from zero-order spectra at 232 nm or from the second derivative of the spectra at 211 nm, while picloram was determined from the first derivative of the spectra at 232 nm. The derivatives of the spectra were smoothed by the adjacent averaging method, using 5 data points for smoothing.

In addition to recording the spectra of the investigated compounds, advantage was taken of the deconvolution mode of the software provided with the spectrophotometer. In this case, calibration was performed with standard solutions of picloram and triclopyr, each having concentration of $13 \mu\text{g cm}^{-3}$. Calibration was followed by recording the spectra of the sample. Subsequently, the concentration of each herbicide in the mixtures automatically appears on the display of the spectrophotometer.

RESULTS AND DISCUSSION

Optimization of working conditions

The derivative spectrophotometric determination of the selected herbicides was preceded by investigations concerning the influence of pH, in the pH interval from 1 to 9. These investigations were carried out individually for each herbicide (Fig. 2). The dependence of the absorption spectra with the medium acidity indicates that picloram and triclopyr are involved in acid–base reactions. It was observed that there were no significant changes in the shape of the spectra in the pH interval from 3.2 to 9.0. All further determinations were carried out at pH 3.2. Bearing in mind that, according to literature data, the pK_a value for picloram is in the interval 2.2–3.6,^{8–10} and for triclopyr 2.68–3.89,¹¹ it can be concluded that dissociation of the carboxyl group occurred at the employed pH value.

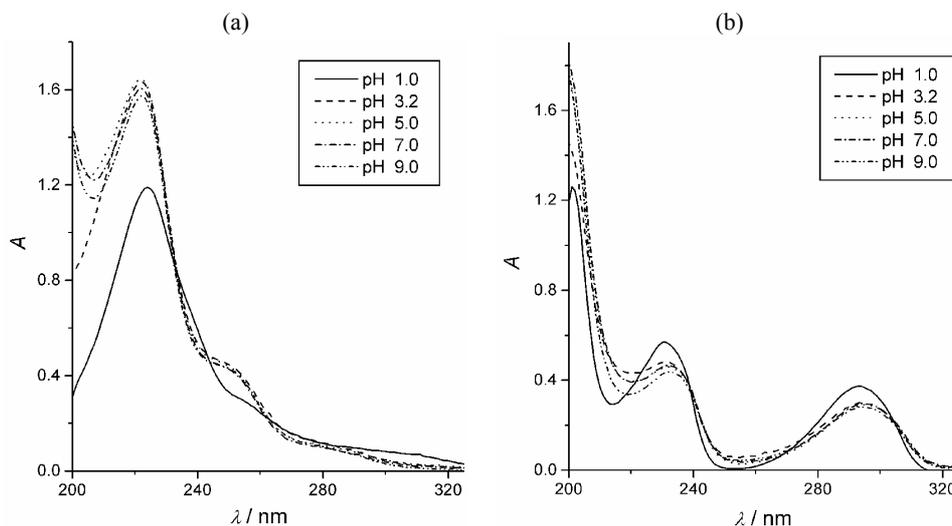


Fig. 2. The influence of pH on the appearance of $12.60 \mu\text{g cm}^{-3}$ picloram (a) and $12.50 \mu\text{g cm}^{-3}$ triclopyr (b) zero-order derivative spectra.

To additionally optimize the working conditions, investigations were undertaken to determine whether the investigated compounds degrade in the presence of light during the time necessary to perform the analysis. Firstly, since there are literature data¹ indicating photodegradation of these herbicides, the spectrum of each compound was recorded five times in a row, in the wavelength region from 200 to 325 nm, to determine if any degradation occurred during the recording of the spectrum. This was found not to be the case, *i.e.*, there were no relevant changes in the peak shape, position or intensity for both herbicides, indicating that no degradation occurred during the recording of the spectrum. Secondly, two series of solutions were prepared with the aim to determine if any degradation of the investigated herbicides occurs under normal laboratory conditions, *i.e.*, at room temperature in day-

light. One series was left to stand in the light, near a window, while the other was the control series which was, for that reason, left in the dark, also at room temperature. No significant changes occurred in either series of solutions within nine months and it can, therefore, be concluded that the analyses can be carried out under normal laboratory conditions, without the use of special working conditions.

Determination

After the preliminary investigations, the absorption spectra of triclopyr and picloram (Fig. 3) were recorded individually and in a mixture in the region between 200 and 325 nm. As a result of the similarity of the structures of the investigated compounds, their absorption spectra partially overlap and the analysis of their mixtures by direct measurement of the absorbance signals was not possible. For this reason, the use of first to fourth order derivative spectra for the determination of each herbicide, as well as their mixtures was tested. One of the frequently used methods in derivative spectrometry is the "zero-crossing" method,^{4-7,12-17} which calls for the determination of one component at the wavelength at which the derivative spectrum of the other passes through zero. This method was used in the present study. Thus, it was found that the use of the first derivative of the spectra at 232 nm (Fig. 4a, curve 1) was optimal for the determination of picloram, while the use of the second derivative of the spectra at 211 nm (Fig. 4b, curve 2) was best suited for the determination of triclopyr, since at these wavelengths practically no interferences were present. The use of higher order derivatives of the spectra (third and fourth derivative) did not give satisfactory results due to the high signal to noise ratio and, for this reason, these derivatives of the spectra were not employed further.

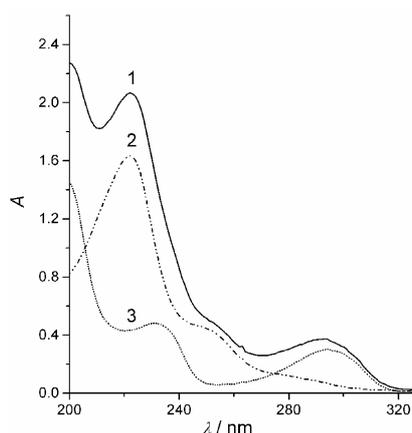


Fig. 3. Absorption spectra of (1) mixture of $12.60 \mu\text{g cm}^{-3}$ picloram and $12.50 \mu\text{g cm}^{-3}$ triclopyr; (2) $12.60 \mu\text{g cm}^{-3}$ picloram; (3) $12.50 \mu\text{g cm}^{-3}$ triclopyr. pH 3.2.

Calibration curves were constructed from the first and second derivatives of the picloram and triclopyr spectra, respectively, at the chosen wavelengths. The regression coefficients and linearity ranges of the calibration curves, obtained by

the linear fit of the acquired data using the least squares method, are presented in Table I. The same table presents the limits of detection (LOD) and quantification (LOQ), which were calculated for both herbicides from the first and second derivatives of the spectra of 20 blank measurements and the slopes of the corresponding calibration curves. Namely, the LOD was determined as the analyte concentration giving a signal equivalent to three times the standard deviation of the blank signal, while for the LOQ this concentration was taken to be equal to ten times the standard deviation of the blank signal.

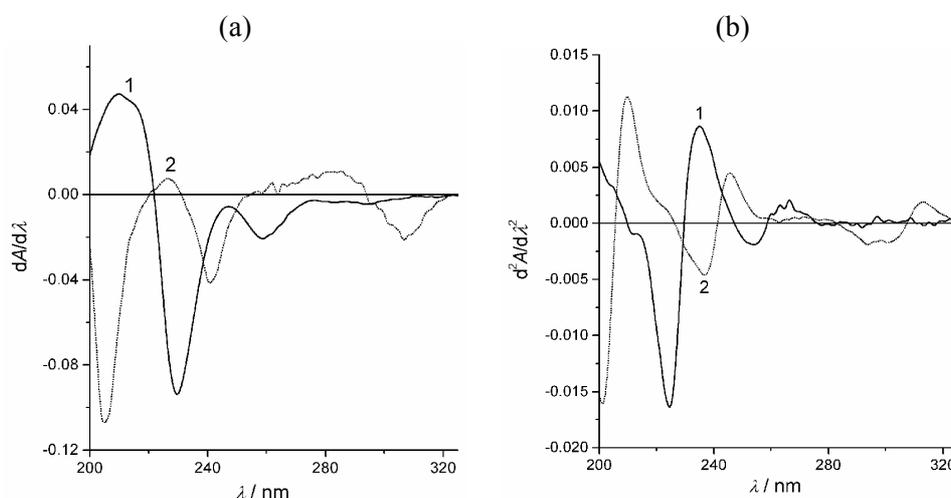


Fig. 4. Derivative spectra of (1) $12.60 \mu\text{g cm}^{-3}$ picloram and (2) $12.50 \mu\text{g cm}^{-3}$ triclopyr: (a) first and (b) second. pH 3.2.

After establishing the optimal working conditions for the determination of the selected herbicides, the developed method was applied to the analysis of three synthetic binary mixtures of picloram and triclopyr. Bearing in mind that triclopyr appears in the commercially available formulations in form of the triethanolamine salt or butoxyethyl ester, and picloram as the hexyloxypropyl amine salt and that they rapidly dissociate/hydrolyse in water to the triclopyr acid/anion,¹⁸ *i.e.*, picloram acid/anion (in dependence on the pH of solution), and triethanolamine, butoxyethanol, *i.e.*, hexyloxypropyl amine, the use of triclopyr and picloram as the analytical standards for the preparation of the synthetic binary mixtures was justified. The composition of these mixtures was chosen to simulate the composition of commercially available formulations. Namely, in commercially available formulations, the ratios of triclopyr and picloram varies from 2:1 to 3:1. Furthermore, the ratios of triclopyr and picloram were chosen with the aim of establishing the influence of the composition of the mixture on the analysis results. The first and second derivatives of the spectra for all three mixtures are presented in Fig. 5 and the results of these determinations together with the accuracy and precision data are presented in

Table II. On comparing the results obtained for the investigated mixtures according to the t -test, it can be seen that there was no significant difference between the taken and found concentration of the analyzed compounds at the 0.05 level, except in the case of picloram in mixture 3, where there was no significant difference at the 0.01 level. It can, thus, be concluded that the applied method can satisfactorily be employed for the determination of the above said herbicides.

TABLE I. Statistical analysis of calibration graphs in the determination of picloram and triclopyr by derivative spectrophotometry method

Parameter	Picloram	Triclopyr	
Derivative	first	zero	second
Wavelength, nm	232	232	211
Concentration range, $\mu\text{g cm}^{-3}$	0.8–13	1.6–13	0.8–13
LOD, $\mu\text{g cm}^{-3}$	0.08	0.04	0.03
LOQ, $\mu\text{g cm}^{-3}$	0.28	0.13	0.11
<i>Regression equation^a</i>			
Slope (b)	-6.76×10^{-3}	5.10×10^{-2}	1.27×10^{-3}
Standard deviation of slope (S_b)	6.72×10^{-5}	1.76×10^{-3}	1.95×10^{-5}
Intercept (a)	-8.40×10^{-4}	1.52×10^{-2}	3.69×10^{-4}
Standard deviation of intercept (S_a)	5.01×10^{-4}	1.38×10^{-2}	1.53×10^{-4}
Standard error of estimations (S_e)	5.84×10^{-4}	1.61×10^{-2}	1.79×10^{-4}
Correlation coefficient (r)	-0.9998	0.9991	0.9996

^a $Y = a + bc$, where c is concentration in $\mu\text{g cm}^{-3}$ and Y is A (zero derivative), $dA/d\lambda$ (first derivative) and $d^2A/d\lambda^2$ (second derivative). The linearity for picloram and triclopyr determined by a six-point calibration.

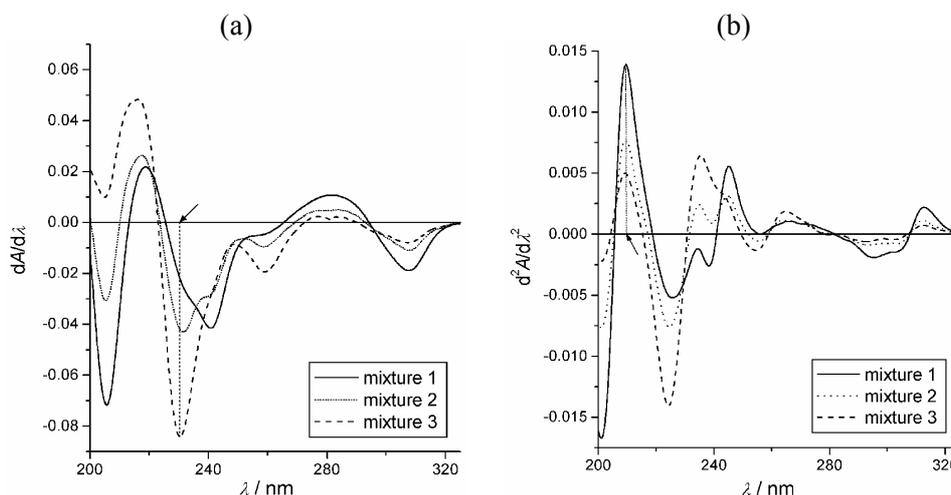


Fig. 5. Derivative spectra for mixtures of picloram and triclopyr: first derivative (a) and second derivative (b). pH 3.2. Mixture 1— $4 \mu\text{g cm}^{-3}$ picloram and $12 \mu\text{g cm}^{-3}$ triclopyr; mixture 2— $6 \mu\text{g cm}^{-3}$ picloram and $6 \mu\text{g cm}^{-3}$ triclopyr; mixture 3— $12 \mu\text{g cm}^{-3}$ picloram and $4 \mu\text{g cm}^{-3}$ triclopyr.

TABLE II. Results of the determination of picloram and triclopyr content in synthetic mixtures ($n = 6$)

Mixture	Found concentration $\mu\text{g cm}^{-3}$		Recovery %		RSD %		t -Test ^a	
	Picloram	Triclopyr	Picloram	Triclopyr	Picloram	Triclopyr	Picloram	Triclopyr
1	4.22	12.28	99.8	100.4	2.51	1.68	0.16	0.52
2	6.38	6.40	100.2	100.4	1.65	0.78	0.33	1.17
3	11.98	4.30	99.3	100.4	0.59	0.69	3.07	1.35

^a $t_{0.05;5} = 2.57$; $t_{0.01;5} = 4.03$.

The same synthetic mixtures were then analyzed by applying the deconvolution mode of the spectrophotometer (Table III). It can be concluded from the results of the t -test, that when the picloram content was determined, in all cases, there was a significant difference at the 0.01 level, while the accuracy was better in the case of the determination of triclopyr, *i.e.*, for the mixture 1 and 3, there was no significant difference at the 0.05 level and for mixture 2, there was no significant difference at the 0.01 level. Therefore, the deconvolution mode of analysis is not entirely applicable to the analysis of the investigated mixtures, *i.e.*, better results are obtained using the developed method.

TABLE III. Results of the determination of picloram and triclopyr content in synthetic mixtures (deconvolution mode of the spectrophotometer) ($n = 6$)

Mixture	Found concentration $\mu\text{g cm}^{-3}$		Recovery %		RSD %		t -Test ^a	
	Picloram	Triclopyr	Picloram	Triclopyr	Picloram	Triclopyr	Picloram	Triclopyr
1	4.42	12.18	104.6	99.5	1.62	1.20	6.68	1.04
2	6.25	6.56	98.1	102.9	1.16	2.27	4.14	3.06
3	11.78	4.35	97.6	101.5	0.89	2.36	6.66	1.51

^a $t_{0.05;5} = 2.57$; $t_{0.01;5} = 4.03$.

The developed method was applied for the determination of triclopyr in Garlon 3A. In this case, the suitability of the zero-order spectra, second derivative spectra and the deconvolution mode were investigated. Namely, since Garlon 3A contains only triclopyr, the possibility of using zero-order spectra for determination of its content was investigated. Statistical analysis of the calibration graph for the determination of triclopyr by the zero-order spectrophotometric method is given in Table I. Since the employed spectrophotometer can also operate in the deconvolution mode, the results obtained by this method were compared to those obtained from the zero-order and second derivative spectra. The results of these determinations are presented in Table IV. From the relative standard deviation (RSD) values, as well as from the results of the t -test, it can be concluded that zero-order and second derivative spectra can be satisfactorily used for determination of triclopyr in Garlon 3A. However, the deconvolution mode is not at all appropriate for the determination of triclopyr in the pesticide formulation.

TABLE IV. Results of the determination of triclopyr content in Garlon 3A ($n = 6$)

Mode of determination	Found ^a g	Recovery %	RSD %	t -Test ^b
Zero-order	31.62	99.42	1.16	1.22
Derivative	31.80	99.99	0.48	0.04
Deconvolution	37.85	119.04	0.62	63.4

^aNominal value for pesticide formulation is 31.8 g per 100 g. ^b $t_{0.05;5} = 2.57$; $t_{0.01;5} = 4.03$.

Furthermore, the developed method was used for the determination of picloram and triclopyr in a mixture made by the addition of picloram to the solution of Garlon 3A. The results of these determinations are presented in Table V. As can be seen, the recoveries obtained from derivative spectra were somewhat higher than 100 % for both determined herbicides. Nevertheless, there was no significant difference between the taken and found concentration at the 0.05 level for picloram, and no significant difference at the 0.01 level for triclopyr. However, in case of the deconvolution method, the results are significantly higher in both cases. It can be concluded that this method is not at all appropriate for the determination of picloram and triclopyr in this type of mixture.

TABLE V. Results of the determination of picloram and triclopyr content in a mixture of Garlon 3A and picloram ($n = 6$)

Mode of determination	Found concentration $\mu\text{g cm}^{-3}$		Recovery %		RSD %		t -Test ^a	
	Picloram	Triclopyr	Picloram	Triclopyr	Picloram	Triclopyr	Picloram	Triclopyr
Derivative	4.14	12.54	101.0	101.3	2.17	0.80	0.33	3.92
Deconvolution	5.48	15.91	133.6	128.5	1.85	0.95	33.3	50.8

^a $t_{0.05;5} = 2.57$; $t_{0.01;5} = 4.03$.

CONCLUSIONS

A derivative spectrophotometric method using the zero-crossing method was developed for the determination of the herbicides picloram and triclopyr in mixtures at pH 3.2. For the determination of picloram, the first derivative was used by measuring the signal at 232 nm, while the second derivative was used for the determination of triclopyr by measuring the signal at 211 nm. The developed method was successfully used to determine picloram and triclopyr in synthetic mixtures, as well as in a triclopyr formulation Garlon 3A and Garlon 3A spiked with picloram, which indicates the possibility of application of the method not only for these mixtures and pesticide formulations, but for many others as well.

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ИЗВОД

ДЕРИВАТИВНО СПЕКТРОФОТОМЕТРИЈСКО ОДРЕЂИВАЊЕ ХЕРБИЦИДА
ПИКЛОРАМА И ТРИКЛОПИРА У СМЕШИБИЉАНА Ф. АБРАМОВИЋ¹, ВЕСНА Б. АНДЕРЛУХ¹, FERENC F. GAÁL^{1,2} и ДАНИЕЛА В. ШОЈИЋ¹¹Природно–мајеминички факултет, Дејарман за хемију, Трџ Д. Обрадовића 3, 21000 Нови Сад и²Војвођанска академија наука и уметности, Дунавска 37, 21000 Нови Сад

Пиклорам (4-амино-3,5,6-трихлорпиридин-2-карбоксилна киселина) и триклопир (3,5,6-трихлор-2-пиридилдоксисирћетна киселина) су селективни системични хербициди, који се у заштити биља примењују за уништавање једногодишњих и вишегодишњих широколисних коро­ва. Како се на тржишту у пестицидним формулацијама могу наћи појединачно и у смеши, у овом раду је развијена деривативна спектрофотометријска метода за њихово селективно одређивање у смеши. У циљу оптимизације одређивања одабраних хербицида, најпре је испитан утицај рН на изглед њихових спектара, у интервалу од 1 до 9 рН јединица. При рН 3,2 раствори пиклорама и триклопира су стабилни на дневној светлости 9 месеци. Сва даља одређивања су вршена при рН 3,2. Нађено је да је за одређивање пиклорама оптимално користити први извод спектра, мерењем сигнала на 232 nm, док је за одређивање триклопира оптимална примена другог извода спектра, мерењем сигнала на 211 nm. Линеаран опсег мерења је од 0,8 до 13 $\mu\text{g cm}^{-3}$ за оба хербицида са коефицијентом корелације од $-0,9998$ за пиклорам и $0,9996$ за триклопир. Граница детекције разрађених поступака је $0,08 \mu\text{g cm}^{-3}$ за пиклорам и $0,03 \mu\text{g cm}^{-3}$ за триклопир. Деривативна спектрофотометријска метода се показала погодном за одређивање пиклорама и триклопира у смеши и у формулацијама пестицида, за разлику од деконволуционе методе.

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