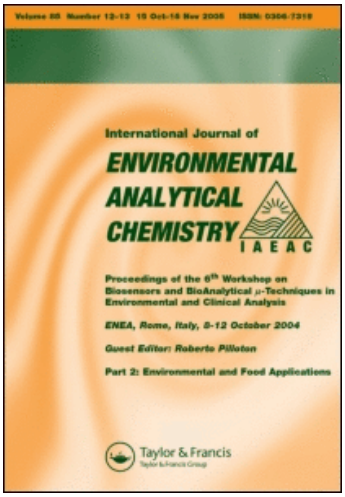

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A Kinetic Spectrophotometric Method to Determine the Insecticide Methyl Parathion in Commercial Formulations and the Aqueous Environment

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A KINETIC SPECTROPHOTOMETRIC METHOD TO DETERMINE THE INSECTICIDE METHYL PARATHION IN COMMERCIAL FORMULATIONS AND THE AQUEOUS ENVIRONMENT

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A simple kinetic-spectrophotometric method for the analysis of the organophosphate insecticide methyl parathion is presented. The method is based on the alkaline hydrolysis of the insecticide into its main metabolite p-nitrophenol. The influence of reaction variables (pH and temperature), and the effect of other pesticides, are discussed. The calibration graphs (initial rate, fixed time, fixed absorbance) were linear from 2 to 30 μg/ml. The precision was calculated for the different methods applied, the relative standard deviation being 6.25% for 4 μg/ml.

The proposed kinetic method can be applied directly to synthetic mixtures, commercial formulations and different aqueous environment, with recoveries close to 100%.

KEY WORDS: Kinetic method, spectrophotometry, methyl parathion, p-nitrophenol, commerical formulations, water analysis.

1. INTRODUCTION

Under certain environmental conditions, the non-systemic organophosphate insecticide methyl parathion (dimethyl p-nitrophenylphosphorothionate) can persist in the ground and the aqueous environment during a few days or weeks 1,2 at μg/ml levels. Its presence in these media makes it a potential hazard because of its high mammalian toxicity as well as that of its major metabolite p-nitrophenol.

Its continuous use in agriculture[3] and the need of a legislation concerning maxima residue limits in the environment, have rised the development of numerous analytical methods. Most methods involve separatory techniques prior to quantitation such as GC,[4–6] TLC,[7–10] GLC,[11–15] LC[16] and HPLC,[2,17–19] with detection limits in the ng/ml levels. TLC is used as an alternative to GC in the analysis of methyl parathion in environmental studies of air, soil, plants or foodstuff.[7,8] However, due to the thermal lability of methyl parathion, the results obtained uing GC are less feasible. Other techniques such as polarography[20–23] or radioimmunoassay[24] gave the best analytical sensitivity.

The detection technique most frequently used has been spetrophotometry. Direct

spectophotometric analysis is always preferred,[25–27] but derivatization reactions using different chromogenic reagents are usually employed.[28]

Although kinetic methods were first employed long ago, in recent years the kinetic approach to quantitative analytical chemistry has been widely used as an alternative to the conventional equilibrium methods of analysis.[29–31] It is important to note that the specifications given by kinetic methods, especially sensitivity and selectivity, depend on the monitoring technique, which could make the kinetic approach particularly attractive and practical.

It is the aim of this paper to show the applicability of the kinetic methodology with UV detection for the analysis of the insecticide methyl parathion in commercial formulations and surface waters. The method is simple and rapid and can compare favourably with those proposed earlier and could be used in routine analysis with a simple equipment required.

2. MATERIALS AND METHODS

Apparatus

All spectra were monitored with a Shimadzu UV-240 Graphicord recording spectrophotometer using quartz cells of 1 cm path length. The cell compartment was thermostatized by circulating water through a jacketed cell holder. A Selecta Univeba-400 water-bath heater was used for temperature control between 0 and 100 °C. Automatic pipettes (Mactrotransferpettor, 1–5 ml, and Microtransferpettor, 0–100 μl) were used for liquid transferation into the test tubes.

The analytical signal changes were recorded at the maximum fixed wavelength of 400 nm on moving chart paper. All the analytical information was obtained from the initial rate measurements.

Reagents

Stock solutions of methyl parathion (min. 99%, Riedel-de-Haen, AG Seelze, Hannover, quality Pestanal) were prepared in acetone (Merck) at 1.32 g/l. Sodium hydroxide 0.5 M and 2 M solutions were of analytical reagent grade (Merck). Redistilled water was used as solvent. Carbaryl, chlortholuron, fenitrothion, promecarb and gusathion were also purchased from Riedel-de-Haen (quality Pestanal, min. 99%) and propham from Serva, Feinbiochemica (99%). They were all prepared in acetone at 1 g/l.

Analytical Procedure

Place different aliquots of the stock solution of the insecticide (1.32 g/l) in 15 ml test tubes to obtain final concentrations between 10–150 μg. To this, add different volumes of NaOH (0.5 or 2 M) to maintain, in all cases, a final volume of 5 ml.

During measurements, keep all standards and reagent solutions at working temperatures (25 °C, NaOH 2 M; 55 °C, NaOH 0.5 M). Start the stop-watch when the last drop of sodium hydroxide solution has been added. Monitor the absorbance of the solution at the optimum wavelength of 400 nm for 4 minutes, starting the recording 30 s after zero time. At this point, the initial rate ($\Delta A/\Delta t$) is calculated.

Sample Preparation

Commercial formulations: Prepare vacuum filtration assembly consisting of 250 ml filter flask, funnel holder, and 30 ml medium porosity fritted glass Bûchner funnel. Add accurately weighed sample containing 1.00 ± 0.01 g of active methyl parathion to Bûchner funnel. Add 10 ml of diethylether to funnel and leave to stand 5 minutes. Apply vacuum when all liquid is in filter flask. Add another 10 ml to funnel and repeat the operation two more times. Release vacuum and disassemble apparatus. Quantitatively transfer contents of filter flask to a round-botton flask and take it to near dryness on a rotary evaporator at 30 °C. To the residue, add 15 ml of acetone, stop it and mix well by inverting several times. Finally, transfer the contents to a volumetric flask and dilute to a total volume of 25 ml with acetone. The final concentration was 40 mg/ml. Aliquots of this solution were used for the analytical determination.

Environmental water samples: For the analysis of run-off water, samples were collected into 250 ml Pyrex containers from three water sources (two streams, and one natural fountain) in Sierra Nevada (Granada, Spain) to give representative samples of surface waters. Samples were stored for no more than 24 h at 4 °C in the dark. Since methyl parathion was not found in the samples at levels above the limit of detection of the procedure (2 μg/ml), microliter amounts of the standard methyl parathion solution in acetone (1.32 g/l) were diluted with the run-off water to give final concentrations between 7–13 μg/ml. Aliquots of 0.5 ml were transferred to 15 ml test tubes and treated as described under analytical procedure.

3. RESULTS AND DISCUSSION

Influence of Reaction Variables

The alkaline hydrolysis of methyl parathion leads to its highly toxic and major metabolite p-nitrophenol.[2,21,33] The reaction is fairly slow (130 minutes at 25 °C), so the possibility of a kinetic-spectrophotometric determination of the insecticide as an alternative to the spectrophotometric method based on the total conversion to its metabolite[27] was taken into account.

For a sensitive determination using spectrophotometry as the indicator technique, it is necessary to obtain a product reaction with a high molar absorptivity. Previous experiments[27] indicate that the transformation takes place when addition of NaOH to the reagent solution is carried out. These results in a yellow coloration of the solution which can be observed by a bathochromic shift of the

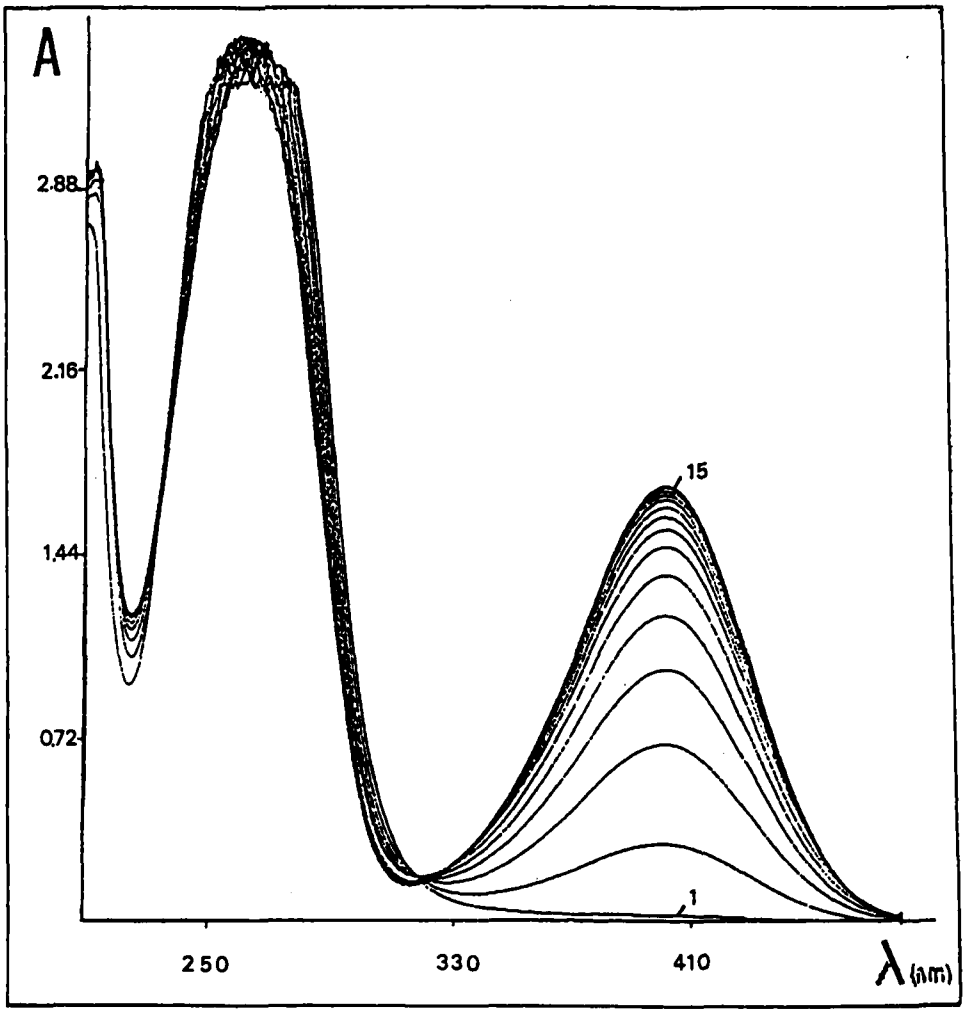

Figure 1 Normal absorption of the hydrolysis process of methyl parathion into p-nitrophenol. [Methyl parathion] = 26.32 μg/ml; [NaOH] = 0.1 M; T = 45 °C. Curve 1: 0 minutes; Curve 15: 45 minutes.

absorption maxima of methyl parathion (270 nm) to that of p-nitrophenol (400 nm), with a molar absorptivity of 17.200 mol/l.cm.

Absorption spectra of methyl parathion and its variation as a function of time is shown in Fig. 1. The hydrolysis reaction rate was determined by monitoring the increase in the absorbance measured at 400 nm at a function of time. This rate is strongly influenced by the sodium hydroxide concentration, reagent concentration and temperature. The effects of these variables on the reaction were studied to obtain the optimum conditions to determine methyl parathion and to establish the kinetic dependence of each of these variables.

The optimum concentration of a species was chosen by changing its initial

concentration, the remaining variables being fixed. The optimum concentrations are those at which the relative standard deviation in the initial rate measurements is minimal. This is found when the reaction order of a species is zero or close to it as possible, since then small fluctuations in concentration will not affect the initial reaction rate as a result of the concentration independence of the initial rate on a zero-order species.

The sodium hydroxide concentration was varied over the range 0.05–5 M at 45 °C and from 1–5 M at 25 °C to investigate its influence on the reaction rate. The initial rate at both temperatures increases with the NaOH concentration with a slight diminution at concentrations greater than 5 M (25 °C) and 1 M (45 °C), respectively. Lower temperatures require higher NaOH concentrations to obtain reasonable rate of methyl parathion hydrolysis.

From these experiments, it is deduced that the reaction is zero-order with regard to the concentration of sodium hydroxide at both temperatures for concentrations between 1 and 3 M. Any NaOH concentration between this interval could be chosen to measure the initial rate reaction in further experiments.

Temperature has also an important influence on the hydrolysis rate. Its effect was studied for the total reaction recording the absorbance/time curves at different temperatures over the range 25–55 °C, at two fixed sodium hydroxide concentrations (0.5 and 2 M). Fig. 2 indicates that temperature influences greatly the initial reaction rate. Both the reaction rate and the absorbance increase with increasing temperature being maxima at 55 °C. With NaOH 0.5 M, there is a gradual increase of both initial rate and absorbance but at NaOH 2 M and temperatures greater than 45 °C, the reaction is too fast and a slight diminution of the absorbance is observed as a consequence of a decomposition process.

From these studies, two pairs of conditions of pH and temperature are suggested to establish the proposed kinetic-spectrophotometric method for methyl parathion:

$$[\text{NaOH}] = 0.5\ \text{M},\ \text{Temperature} = 55\ ^\circ\text{C}$$

$$[\text{NaOH}] = 2\ \text{M},\ \text{Temperature} = 25\ ^\circ\text{C}$$

A normal room temperature such as 25 °C has been chosen to make the method as operative as possible for those situations where the thermostatization is not possible. Temperatures greater than 55 °C have not been studied to consider that the method would not be operative and the reaction too rapid to be followed.

The kinetic data, obtained by plotting logarithm of initial rate vs. logarithm of concentration of the species, (sodium hydroxide and methyl parathion concentration) gives a curve whose slope at a given concentration is equal to the reaction order with regard to such species. In this case, the reaction is first-order in the range 2 to 7 μg/ml and 7 to 20 μg/ml at 25 °C and between 13 and 30 μg/ml at 55 °C.

Analytical Parameters

To study the concentration effect of the compound to be determined, methyl

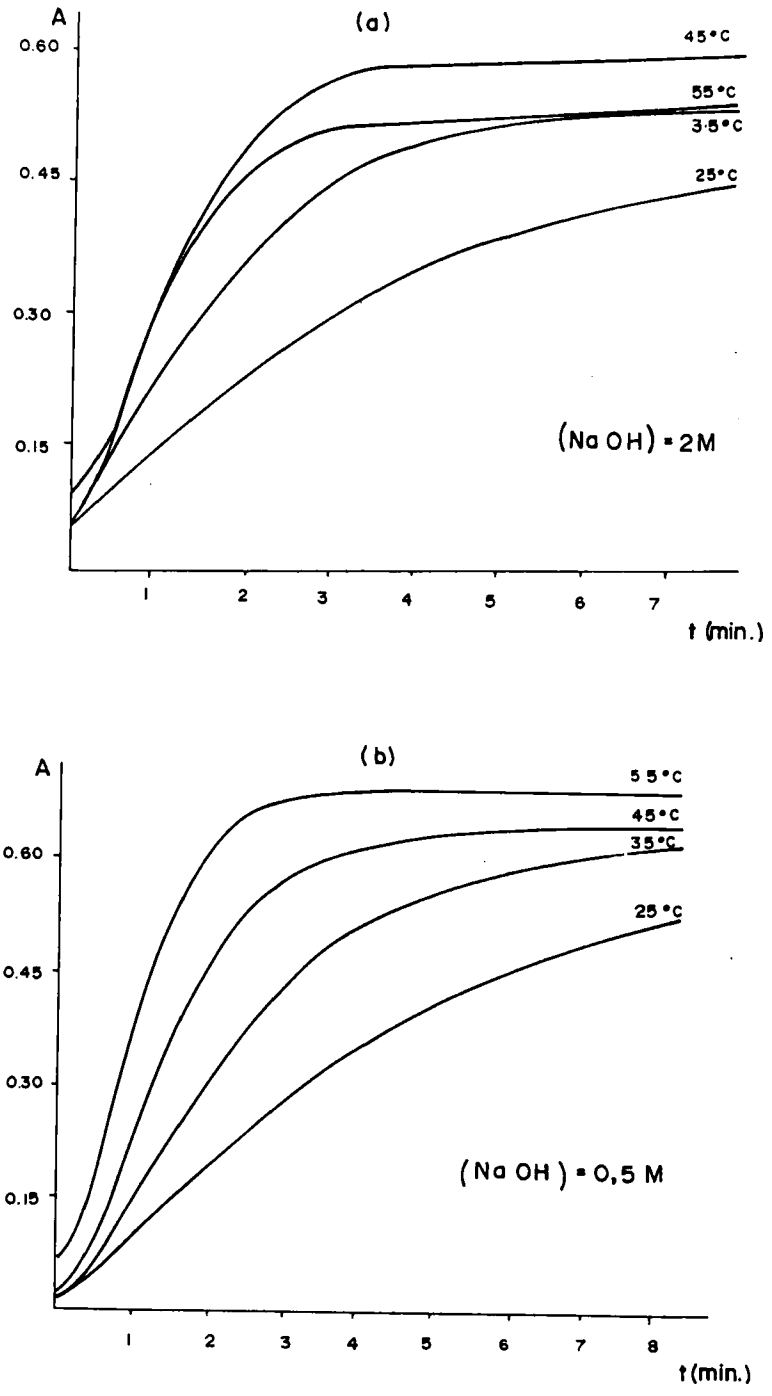

Figure 2 Absorbance/time curves at two NaOH concentrations (0.5 and 2 M) and different temperatures. [Methyl parathion] = 10 μg/ml.

Table 1 Analytical parameters of the kinetic-spectrophotometric method for methyl parathion

Proposed method	*Measurement method*	*Linear range μg/ml*	*Equation*	*Correlation coefficient*
	Initial rate	2–13	$\log tg\alpha = 1.49 \log C - 3.32$	0.9709
		2–13	$A = 0.04C - 0.02$	0.9855
25 °C	Fixed time	10–30	$A = 2.98C - 7.40$	0.9924
		2–13	$\log 1/T = 1.53 \log C - 2.06$	0.9993
	Fixed absorbance	10–30	$\log 1/T = 1.00 \log C - 1.62$	0.9290
		2–13	$\log tg\alpha = 0.88 \log C - 1.48$	0.9882
	Initial rate	10–30	$\log tg\alpha = 1.03 \log C - 1.94$	0.9924
		2–13	$\log A = 0.88 \log C - 0.89$	0.9934
55 °C	Fixed time	10–30	$A = 0.05C + 0.06$	0.9987
		2–13	$1/T = 0.14C + 1.44$	0.9982
	Fixed absorbance	10–30	$\log 1/T = 1.46 \log C - 2.56$	0.9963

parathion, different curves absorbance as a function of time at the maximum wavelength of 400 nm have been recorded over the range 2 to 30 μg/ml at the two pairs of conditions previously fixed.

To obtain the calibration graphs, three methods were employed: (a) the initial rate method, by calculating the slopes ($tg\alpha$) of the plots of absorbance *vs* time for different methyl parathion concentrations; (b) the fixed time method, by measuring the absorbance after 3 minutes of reaction; and (c) the fixed absorbance method, by measuring the time required for the reaction to reach a fixed absorbance value, depending on the experimental conditions. The equations of the different calibration graphs together with other analytical parameters are summarized in Table 1. It can be deduced from these values that the correlation between concentration and signals at 55 °C in all concentration ranges are better than that obtained at 25 °C. For such a reason, the method being carried out at 55 °C is further recommended.

The theoretical detection limit defined as the minimum detectable quantity have resulted to be 2 μg/ml methyl parathion in both conditions, although more accurate results are obtained at 55 °C.

Due to the fact that two different experimental conditions are proposed, the accuracy and precision of the analyses have been determined at 25 °C and 55 °C, with 11 replicates of samples containing 4, 10, 20 μg/ml of methyl parathion, respectively. The results, expressed as the relative standard deviation and relative error for the different methods, indicate that the smaller values are obtained for 10 μg/ml at 55 °C being 3.31 % and 2.22 %, respectively.

In general, both values are smaller for the initial rate, fixed time and fixed absorbance method, except for the lower concentration level of 4 μg/ml where the fixed time method give the best results.

Interference Studies

There could be different ways in which other agricultural chemicals might interfere with the proposed kinetic method but, especially those which can decrease the

Table 2 Interference study. Initial rate method at 55 °C, NaOH = 0.5 M

Interferent[a]	*Concentration found, μg/ml*	*Error, %*
Carbaryl (100)	3.78	−5.5
Chlortholuron (100)	3.63	−9.2
Fenitrothion (4)	4.20	+5.0
Propham (100)	4.17	+4.2
Promecarb (100)	3.55	−11.2
Gusathion (20)	4.20	+5.0
Carbaryl (100) + Chlortholuron (100)	3.63	−9.2
Carbaryl (100) + Fenitrothion (1)	4.17	+4.2
Propham (100) + Gusathion (20) + Fenitrothion (1)	4.17	+4.2
Promecarb (100) + Gusathion (20) + Propham (50)	4.17	+4.2

[a]Concentration methyl parathion added = 4 μg/ml; In parenthesis, concentration in μg/ml.

initial concentration of NaOH via their own alkaline hydrolysis, would be expected to be sources of interference.

To evaluate the selectivity of the analytical method, having this in mind, a number of commonly occurring pesticides in natural waters were examined. The effect of the insecticides carbaryl, gusathion, fenitrothion, promecarb and the herbicides chlortholuron and propham, under the conditions described for analysis, were investigated.

The results obtained when applying the initial rate method on the determination of 4 μg/ml methyl parathion in presence of 1,2 or 3 interferents are presented in Table 2, using a tolerance criterion of $x \pm 2s_A$.[34]

The fixed absorbance and fixed time methods were also applied, but the results were not within the tolerance criterion selected. Thus, the initial rate method is considered the most selective when carried out at 55 °C and 0.5 M sodium hydroxide. Table 2 indicates that it is possible a 25:1 concentration ratio (maximum ratio tested) for carbaryl, chlortholuron, propham and promecarb; 5:1 for gusathion and no greater than 1:1 for fenitrothion with positive and negative errors within the tolerance criterion range.

Analysis of Methyl Parathion in Commercial Formulations and Environmental Water Samples

The highly toxic agricultural chemical methyl parathion is used in large quantities for insect control on cotton and other plants. In Spain, methyl parathion is part of numerous commercial formulations alone or in mixtures with other pesticides as phosalone, ethyl parathion, malathion, etc ...

FOLIDOL M-2 and PROBEL MP-2 insecticides are two of these formulations containing methyl parathion as a 2% active ingredient, and are presented as wettable powders. Methyl parathion was extracted from the commercial formulations by the cleanup technique of Sane[25] to demonstrate the applicability of the present method to control their commercial specifications. The results are shown in Table 3.

For this determination, the samples were measured by the initial rate, fixed time

Table 3 Analysis of methyl parathion in different commercial formulations

Commercial formulation	*Sample No.*	*Initial rate method*				*Fixed time method*			
		25 °C		*55 °C*		*25 °C*		*55 °C*	
		(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
	1	3.16	1.58	2.19	1.10	7.25	3.60	4.07	2.03
PROBEL MP-2	2	3.31	1.65	2.57	1.30	7.00	3.50	4.36	2.18
	3	3.02	1.51	2.57	1.30	7.00	3.50	3.80	1.90
$x \pm s_x$		3.16 ± 0.14	1.58 ± 0.07	2.40 ± 0.20	1.23 ± 0.10	7.10 ± 0.14	3.50 ± 0.06	4.07 ± 0.28	2.04 ± 0.14
	1	3.98	1.99	2.51	1.25	5.75	2.90	3.09	1.54
FOLIDOL M-2	2	3.31	1.65	2.57	1.28	5.50	2.70	3.23	1.61
	3	3.80	1.90	2.82	1.41	6.00	3.00	3.23	1.61
$x \pm s_x$		3.70 ± 0.30	1.85 ± 0.17	2.60 ± 0.20	1.30 ± 0.08	5.75 ± 0.25	2.80 ± 0.10	3.18 ± 0.08	1.60 ± 0.04

[a] Concentration of methyl parathion found in μg/ml.

[b] Percentage methyl parathion found in the commercial formulation. Label declaration = 2%.

Table 4 Analysis of methyl parathion in different environmental water samples

Water[a] *Samples*	*Concentration added, μg/ml*	*Recovery, %*[b] *Initial rate method*		*Fixed time method*	
		25 °C	*55 °C*	*25 °C*	*55 °C*
	7	147.28	116.36	123.28	103.19
1	10	117.75	104.50	116.80	97.37
	13	118.38	90.54	121.15	95.64
	7	140.57	115.00	117.00	83.14
2	10	121.80	100.03	121.50	77.60
	13	119.61	95.77	117.54	78.85
	7	152.28	115.14	113.57	99.00
3	10	127.10	104.70	126.40	95.90
	13	128.77	94.38	122.08	93.38

[a]Type of water: 1, River Darro, Sierra de Alfacar, 1300 mts.; 2, Fountain of D. Manuel, Sierra Nevada, 1900 mts.; 3, River Genil, Sierra Nevada, 1200 mts in Granada (Spain).
[b]Mean value of three extractions and three determinations.

and fixed absorbance methods, but those obtained with the latter are not within the confidence limits established, and the results have not been considered. The method carried out at 55 °C give the best results by both measurement methods but at 25 °C, the fixed time method give precise but not accurate results (average value of 3.15% active ingredient, respect to 2% of the label specification).

As it has been indicated previously, under field application conditions, methyl parathion could be expected to leave residues on the crops where is is applied and can enter in surface waters draining the cropland.

To evaluate the applicability of the proposed kinetic method in natural water samples, several different types of water from streams and natural fountains of Sierra Nevada (Granada, Spain) were analyzed. The various water samples were spiked with methyl parathion at several levels between 7 and 13 μg/ml and analyzed as described. Typical quantitative results when applying the initial rate and fixed time method are shown in Table 4.

Potential interference by other agricultural chemicals and organic compounds naturally occurring in the water samples were not observed since none of the water samples gave anomalous absorbance signals which interfered with the determination of the pesticide, under the conditions described.

4. CONCLUSIONS

The kinetic approach using spectrophotometry as the detector technique have proved to be a useful alternative to the conventional equilibrium method for the analysis of the highly toxic organophosphorous insecticide methyl parathion. The alkaline hydrolysis transformation carried out at two temperatures and two different sodium hydroxide concentrations have permitted to establish an easy method suitable to those laboratories which are not equipped with sophisticated instruments.

The good results obtained for the analysis of methyl parathion in a wide variety of samples (synthetic mixtures, commercial formulations and environmental water samples) demonstrated the applicability of the method for routine analysis.

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