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Stopped-flow and kinetic-fluorimetric determination of quinalphos in water samples

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Abstract

The hydrolysis of the pesticide quinalphos in basic medium was kinetically followed and the measurement of the reaction rates allowed us to develop two kinetic-fluorimetric methods. In one of them the mixing of the reagents was directly performed in the measurement cell and, in the another one, the stopped-flow mixing technique was used. The reaction was completed in 100 s after the reactants were mixed and it allowed the simple application of the proposed methods to routine analyses of the pesticide. The sensitivity of the methods was very high, being the detection limits 50 and 140 ng mL^{-1} for the manual procedure and the stopped-flow mixing technique, respectively. Both methods were compared using regression with uncertainties in both axes. The effect of the presence of several pesticides in the determination was tested. A solid-phase extraction process was also developed for the application of the methods to diverse waters samples. The proposed kinetic-fluorimetric methods were applied to the determination of quinalphos in drinking water, well water and river water, with very satisfactory results.

Keywords: Quinalphos; Hydrolysis; Fluorescence; Kinetic measurements

1. Introduction

Due to the expansion of the crops, chemical pesticides have been widely used against a great variety of plagues and serious environmental pollution problems have arisen. Thereby, much emphasis is now placed on monitoring the levels and effects of pesticides in air, water and food supplies.

Quinalphos (*O*,*O*-diethyl-*O*-quinoxalin-2-yl-phosphorothioate) is an important organophosphorus pesticide which is used against a great variety of plagues in cotton, vegetables, fruits, etc. This insecticide acts by ingestion and contact and it is characterized by its penetrating action, being its persistence estimated in 10–15 days.

Several methods have been proposed for determining quinalphos in fruits and vegetables, mainly chromatographic methods and, specifically, gas chromatographic methods [1–7]. They are not very suitable for quinalphos analysis because this compound is very sensitive to heat and is decomposing on the column, leading to inaccurate results. Other methods involve liquid chromatography [8,9]. Also, methods without previous separation steps have been developed, such us spectrophotometric [10] and spectrofluorimetric methods [11]. A gravimetric method for determining quinalphos, based on its reaction with copper(I) chloride, has been also proposed [12].

Spectrofluorimetry offers excellent detection limits in the determination of trace amounts of many organic molecules. In this paper, two kinetic-fluorimetric methods have been developed for quinalphos quantification, based on the hydrolysis of this pesticide in alkaline medium. To make the kinetic measurements, the stopped-flow mixing reactor and the manual mixture of the reagents in the cell have been used. In both cases, the reaction rates have been obtained by using two different methods. Finally, a previous process of extraction has been optimized in solid-phase, with the object of determining quinalphos in diverse water samples. These procedures have been satisfactorily applied to the determination of the pesticide in drinking water, well water and in water of the Guadiana River to its passage through the city of Badajoz (Spain).

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2. Experimental

2.1. Reagents

All experiments were performed with analytical reagent grade chemicals. Ethanolic standard solutions of 100 μg mL^{-1} of quinalphos (purity 99.5%, Dr. Ehrenstorfer GmbH) were prepared and stored in the dark at 4 °C. Solutions of lower concentrations were prepared by appropriate dilution of the stock solution with ethanol. Sodium hydroxide (Panreac) solutions were prepared by dilution in ultra pure grade water. Sep-Pak Plus C18 Cartridges (360 mg) (Waters) were also used.

2.2. Apparatus and software

The acquisition of kinetic data and the fluorescent measurements were made on a SLM Aminco Bowman, Series 2 luminescence instrument, equipped with a 150 W continuous Xenon lamp, interfaced by a GPIB card and driver with a PC Pentium II microcomputer. Data acquisition was performed by the use of AB2 Software Version 1.40, running under Windows 98.

The instrument incorporates a MilliFlow stopped-flow reactor, allowing the study of changes in luminescence reactions, when two reactants are vigorously forced through the mixing chamber and suddenly stopped in the observation cell. The MilliFlow consists of two fill syringes, two drive syringes, an observation cell (path length of 2 mm), a stop syringe, a stop block, and an exhaust and fill valve levers. Hamilton gasting syringes of 2.5 mL (drive syringes) were used to hold the two reactant solutions. The syringes are made from controlled, inner-diameter borosilicate glass with precision machined teflon plunger tips (these pistons are simultaneously driven by air-operated plungers). Thermostatic equipment permits a constant temperature between 10 and 45 °C in the stopped-flow module and in the cell compartment, by circulating water from a Selecta Unitronic 320 OR thermostatic bath.

The kinetic curve processing was performed by means of the ESCIN program, developed by us, in Mat Lab code, which allows the linear region optimization in the kinetic curve, and fit by means of least squares regression to obtain the maximum rate of the reaction. The statistical analysis was performed by means of the ACOC program, developed by us, in Mat Lab code [13].

2.3. Procedures for the kinetic-fluorimetric determination of quinalphos

2.3.1. Manual procedure

An aliquot of the quinalphos in water:ethanol (95:5, v:v) stock solution or sample was transferred to the cell, for a final concentration between 0.067 and 1.2 μg mL^{-1}. Then ethanol, if necessary, to complete 5%, deionized water to complete 2.0 and 1.0 mL of 4.5 M NaOH solution were added. The evolution of the fluorescence emission intensity with time at 418 nm (λ_{ex} = 353 nm, $bandpass_{ex/em(nm/nm)}$ = 4/8) was scanned during 70 s, with a resolution of 1 s, and maintaining the temperature at 60 °C. The reaction rate was measured as the tangent in the linear part of the kinetic curve obtained by linear regression of 50 experimental points, using a fixed-time interval between 10 and 60 s, or a variable-time interval (Δt = 50 s). The variable-time interval giving the maximum rate was selected. Each sample was assayed in triplicate.

2.3.2. Stopped-flow procedure

One-drive syringe was filled with a solution containing a standard or sample solution of quinalphos in water:ethanol (95:5, v:v) at a final concentration between 0.12 and 5.8 μg mL^{-1}. The other syringe was filled with a solution of NaOH 3 M. Then, the two solutions were mixed in the mixing chamber in each run. The variation of the fluorescence emission intensity with the time was monitored at 418 nm (λ_{ex} = 353 nm, $bandpass_{ex/em(nm/nm)}$ = 8/16), at 25 °C and for 30 s, with a resolution of 1 s. The reaction rate was determined at a fixed-time interval between 10 and 20 s, or at a variable interval time (Δt = 10 s). The variable-time interval giving the maximum rate was selected. The samples were prepared in triplicate and, of each one of them, three injections were made.

2.3.3. Determination of quinalphos in water samples

For the determination, 50 mL of sample (river water, drinking water or well water) were passed through a C18 cartridge, previously conditioned with 10 mL of methanol and 10 mL of deionized water. For the elution, 5 mL of diethyl ether were passed through the cartridge. The eluate was evaporated to dryness and the residue was dissolved in 10 mL of ethanol:water (5:95, v:v). The samples were prepared in triplicate and, of each one of them, three injections were made.

3. Results and discussion

The reaction rates were obtained by using two different methods. In the first method, the fluorescence data in a fixed-time interval was used, selected by application of the least squares method and, in the second case, a variable-time method for selecting the most favourable reaction rate, was applied. In both cases, the ESCIN program was used.

For the manual procedure, the most favourable statistical parameters were obtained in the fixed-time interval between 10 and 60 s and, when the variable-time method was used, an interval of 50 s was selected.

For the stopped-flow procedure, the most favourable statistical parameters were obtained in the range between 10 and 20 s, in order to calculate the reaction rate in a fixed-time interval. To calculate the greatest reaction rate, a time interval of 10 s was selected.

All the reported concentrations, for the stopped-flow procedure are the initial concentrations in the syringes (twice the actual concentrations in the reaction mixture, at time zero after mixing). Each of the kinetic results was the average of three measurements.

3.1. Kinetic-fluorimetric study of quinalphos

Quinalphos is a weakly fluorescent compound, but the hydrolysis of this pesticide in alkaline medium generates quinoxalin-2-ol, which is strongly fluorescent and, by means of the measurement of the reaction rate, two kinetic-fluorimetric methods have been developed for its quantification. Fig. 1 shows the excitation and emission spectra of quinalphos and its hydrolysis product in ethanol/water medium.

The intensity of fluorescence increases when decreases the percentage of ethanol in the medium and, in addition, slight displacements in the maxima of excitation and emission take place. When a 5% (v/v) ethanol/water solution of quinalphos was treated with 1.5 M NaOH, the excitation spectrum of quinoxalin-2-ol shows a maximum located at 353 nm, and the emission spectrum shows a maximum at 418 nm. These were the selected wavelengths to carry out the measurement of the evolution of the fluorescence emission with time.

The influence of the ethanol percentage was studied in the range between 5 and 50% (2.5 and 25% for the stopped-flow procedure, due to dilution in the cell of measurement). It was found that the reaction rate remains practically constant until a value of 15%, and decreases for higher ethanol percentage (−1 partial order). An ethanol percentage of 5% for the manual procedure and for the stopped-flow procedure was selected as optimum.

The effect of the temperature on the reaction rate was examined between 10 and 70 °C, for the manual procedure, and between 10 and 45 °C, for the stopped-flow procedure. In both methods, the rate of the reaction is strongly favoured, and it increases exponentially with the temperature. A temperature of 60 °C was selected for the manual procedure and 25 °C for the stopped-flow procedure, as in this case, higher temperatures are not advisable, because of the serious inconvenience of formation of bubbles.

The study of the influence of NaOH concentration in the manual method, shows that the reaction rate first increased with NaOH concentration until reaching a constant value. In Fig. 2, the variation of the rate of reaction with the concentration of NaOH, is represented. For [NaOH] < 1.3 M, the rate of reaction increased when the concentration increased (1/2 partial order). For higher concentrations, the reaction rate remains about constant. A 1.5 M (within the constant range) concentration of NaOH was selected as optimum to obtain the highest sensitivity.

The study of the influence of NaOH concentration, on the stopped-flow procedure at 25 °C, shows that the reaction rate increased with NaOH concentration in all the range assayed between 0 and 3 M. For higher temperature values (40 °C), the reaction rate was independent of the NaOH concentration for values greater than 2 M NaOH. We have to take into account that a temperature of 25 °C has been selected as, for temperatures higher than 25 °C, the analytical signal is not reproducible due to the instrumental noise and to the formation of bubbles. More concentrated NaOH solutions could attack some components of the stopped-flow reactor. In addition, processes of decomposition of our product could be produced. In consequence, a concentration of NaOH 3 M has been selected.

A plot of the logarithm of the reaction rate, against the inverse of the absolute temperature, allows us to calculate a value of the activation energy of 20 KJ mol^{-1} K^{-1}.

For the manual procedure, the influence of the addiction order was studied and no differences were found.

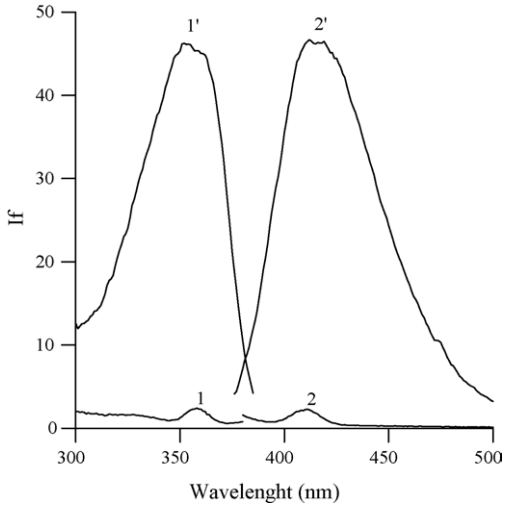

Fig. 1. Excitation and emission spectra of quinalphos (1, 2) and its hydrolysis product (1′, 2′) in ethanol:water (5:95, v/v) [quinalphos] = 1 μg mL^{-1}.

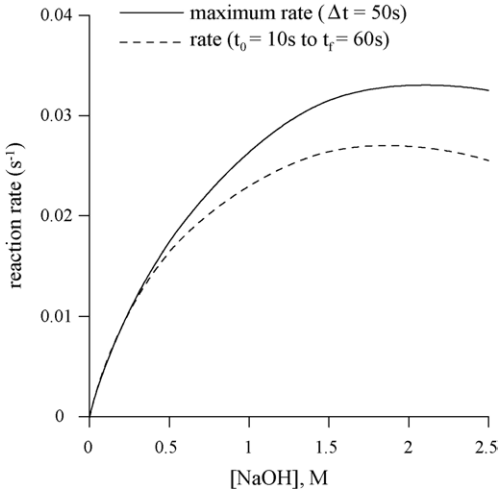

Fig. 2. Influence of NaOH concentration in the reaction rate, for the manual procedure. [Quinalphos] = 1 μg mL^{-1}, maximum rate measured (Δt = 50 s), rate measured between t_0 = 10 s and t_f = 60 s.

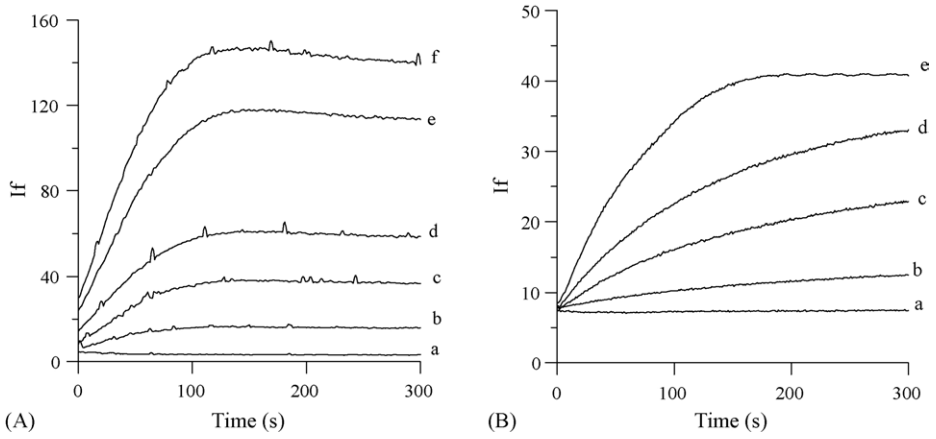

Fig. 3. Kinetic curves recorded for samples containing several concentrations of quinalphos (A) between 0.05 and 1.0 μg mL^{-1}, by the manual procedure (a, 0 μg mL^{-1}; b, 0.067 μg mL^{-1}; c, 0.17 μg mL^{-1}; d, 0.26 μg mL^{-1}; e, 0.50 μg mL^{-1}; f, 0.67 μg mL^{-1}) and (B) between 0.12 and 3.5 μg mL^{-1}, by the stopped-flow procedure (a, 0 μg mL^{-1}; b, 0.12 μg mL^{-1}; c, 0.35 μg mL^{-1}; d, 0.60 μg mL^{-1}; e, 1.16 μg mL^{-1}).

3.2. Calibration curves and analytical parameters

Under the optimum physico-chemical selected conditions, the fluorescence-time signals at 418 nm (λ_{ex} = 353 nm) were recorded between 0 and 300 s, for solutions containing different amounts of quinalphos by the manual technique and by the stopped-flow technique, Fig. 3. The reaction rate values were calculated at fixed-time intervals, between 10 and 60 s, and between 10 and 20 s, for the manual and the stopped-flow techniques, respectively. When a variable-time interval method was used to calculate the reaction rate values, intervals of 50 and 10 s were selected, for the manual and the stopped-flow techniques, respectively. In Table 1, the analytical and statistical parameters of the determination procedures, are summarized. As can be observed, the sensitivity of the manual procedure is better than the stopped-flow method, as in the manual method we can work at higher temperature (60 °C) than in the stopped-flow method, in which, due to the instrumental and chemistry characteristics, we cannot work at temperatures higher than 25 °C.

Under the optimum working conditions, the initial slope of the kinetic curves were consistent with a first-order dependence on the quinalphos. The simplified kinetic equation can be expressed as:

$$\mathrm{d[quinalphos]}/\mathrm{d}t = k[\text{quinalphos}], \quad \text{for [NaOH]} > 1.3\,\text{M} \quad \text{and} \quad [\text{EtOH}] < 15\%$$

The time of measure per sample is 70 s for the manual method and 30 s for the stopped-flow method.

3.3. Influence of foreign species

To evaluate the selectivity of the method, the effect of some other pesticides in the determination of quinalphos was studied. These pesticides (methyl parathion, ethyl parathion, fenitrothion, chlorpyrifos, methidathion and simazine) appear together with quinalphos in citrus fruits, grapes and commercial formulations. As quinalphos, these pesticides are hydrolyzed under suitable conditions of pH and temperature.

Table 1
Analytical and statistical parameters for the kinetic-fluorimetric determination of quinalphos

	Manual procedure		Stopped-flow procedure	
	Fixed-time interval (10–60 s)	Variable-time interval (Δt = 50 s)	Fixed-time interval (10–20 s)	Variable-time interval (Δt = 10 s)
Linear range (μg mL^{-1})	0.050–1.0	0.050–1.2	0.12–3.5	0.12–5.8
Slope	2.02 ± 0.03	2.07 ± 0.02	0.359 ± 0.006	0.394 ± 0.004
Intercept	−0.002 ± 0.01	0.03 ± 0.01	−0.01 ± 0.009	−0.02 ± 0.01
Correlation coefficient	0.999	0.999	0.999	0.999
Relative S.D. (%), n = 10	5.5 (0.87 μg mL^{-1})	6.7 (0.87 μg mL^{-1})	0.8 (1.30 μg mL^{-1})	1.0 (1.30 μg mL^{-1})
LOD (μg mL^{-1})[a]	0.05	0.05	0.14	0.20
LOD (μg mL^{-1})[b]	0.02	0.02	0.06	0.08
Analytical sensitivity[c]	0.02	0.02	0.06	0.09

[a] Clayton et al. ($\alpha = \beta$ = 0.05) [14].
[b] Long and Winefordner (k = 3) [15].
[c] Cuadros Rodríguez et al. [16].

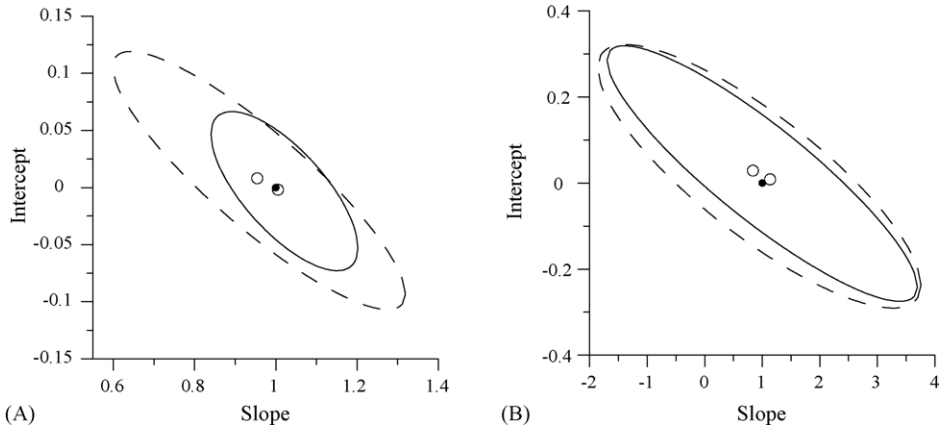

Fig. 4. Joint confidence intervals based on BLS to compare the abilities of the proposed methods. Empty points indicate the calculate point of intercept and slope while the solid ones indicate the theoretical point of zero intercept and unity slope. (A) Comparison of the predictions obtained using the maximum rate at variable-time interval and the reaction rate in a fixed-time interval: for the stopped-flow technique and for the manual procedure. (B) Comparison of the predictions obtained using the stopped-flow and the manual procedure: using the maximum rate at variable-time interval and using a fixed-time interval.

These were tested in proportions regarding the quinalphos (w/w) between 1:1 and 100:1. The same results were obtained for the manual procedure and the stopped-flow technique, calculating the slopes by means of both ways already described for each method. Methidathion and simazine are tolerated at least in a 100-fold excess (w/w) and the others are tolerated at least in five-fold excess.

3.4. Method comparison

These two analytical methods (the manual procedure and the stopped-flow technique) were compared by application of a linear model based on the bivariate least squares (BLS) calibration method, and taking into account the comparable errors in both axes. The results should fit a straight line where the intercept is not significantly different from zero and the slope not significantly different from one. To check these premises, the joint confidence interval for the intercept and the slope was applied [17,18]. Fig. 4 shows the joint confidence intervals for the intercept and the slope for the two methods, which is elliptical in shape. The level of significance chosen was $\alpha = 0.05$. It can be seen that, in all cases, the theoretical point (1, 0) is within the joint interval of the ellipse, which is indicative that these methods give comparable results.

3.5. Determination of quinalphos in water samples

Previous to the determination of this analyte in different water samples, a solid–liquid extraction procedure has been optimized. A Sep-Pak Plus C18 (360 mg) cartridge was used and it was conditioned with 10 mL of methanol and with 10 mL of ultrapure water at an speed of 1.5 mL min^{-1}. Single solvents (such as ethanol, diethyl ether and dichloromethane) and mixtures of two solvents (different proportions of water:ethanol and ethanol:ether) were assayed for the elution step. The results in Table 2 show that diethyl ether is more effective than either dichloromethane or different proportions of ethanol:ether. Other variable that has been studied is the influence of ethanol percentage in the sample. In this case, 10 mL of quinalphos solutions, with ethanol percentages between 5 and 75%, were passed through the cartridge, previously conditioned, and the fractions were analyzed. The percentage recoveries show that, when the percentage was smaller than 30%, the analyte was totally retained and the retention decreased when the percentage of ethanol in the sample increased. The influence of the pH of the sample was studied between 2.5 and 7.5, and the retention of the quinalphos was not affected in this interval.

Table 2
Effect of the solvent, at the elution stage, in the solid-phase extraction of quinalphos, [quinaphos] = 1.0 μg mL^{-1}

Solvent	Recovery (%)
Water:ethanol (70:30, v:v)	7
Water:ethanol (30:70, v:v)	36
Ethanol	64
Ethanol:diethyl ether (70:30, v:v)	66
Ethanol:diethyl ether (30:70, v:v)	76
Ethanol:diethyl ether (20:80, v:v)	77
Diethyl ether	85
Dichloromethane	71

The extraction procedure optimized, summarized in Section 2.3.3, has been applied to the determination of quinalphos in water samples. A portion of 50.0 mL of water sample containing between 0.042 and 0.66 μg mL^{-1} of quinalphos, was passed through the cartridge and eluted with 5 mL of diethyl ether. The eluate was evaporated until dryness and the residue was dissolved in 10.0 mL of water:ethanol (95:5, v:v). When the manual procedure was applied, an aliquot of 2.0 mL was transferred to the cell and the proposed method was applied. When the stopped-flow procedure was applied, one of the syringe was filled with the solution and the proposed method is applied. The obtained results by the first method, in the analysis of samples of drinking water,

Table 3
Results obtained by application of the kinetic-fluorimetric method, manual procedure, in water samples

Sample	Quinalphos added ($\mu g\,mL^{-1}$)	Recovery (%)[a] ± RSD	
		Fixed-time interval	Variable-time interval
Drinking water	0.04	80 ± 5	95 ± 5
	0.10	93 ± 5	96 ± 2
	0.22	87 ± 2	87 ± 1
Well water	0.04	98 ± 1	110 ± 10
	0.10	82 ± 5	87 ± 4
	0.22	80 ± 5	83 ± 1
River water	0.04	94 ± 6	102 ± 7
	0.10	80 ± 6	83 ± 4
	0.22	90 ± 6	88 ± 6

[a] Mean of three determinations.

well water and in water of the Guadiana River, that were polluted with known concentrations of quinalphos, are shown in Table 3 , where it is observed that the recoveries are quite acceptable. Similar results were obtained by the stopped-flow procedure.

4. Conclusions

The hydrolysis of the pesticide quinalphos in basic medium was studied by kinetic procedures, scanning the evolution of the fluorescence signal with the time, mixing manually the reagents in the cell, and using a pneumatic stopped-flow module. The form of measuring the reaction rates, using a fixed-time interval or a variable-time interval does not considerably influence the results. The detection limit is better in the manual method but the precision of measurements is strongly improved by using the pneumatic stopped-flow module. A solid-phase extraction was optimized for the determination of quinalphos in water samples, and satisfactory results were obtained in the determination of the pesticide, at $\mu g\,mL^{-1}$ levels, in diverse water samples, obtaining recoveries between 80 and 110% in all the cases.

Acknowledgements

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