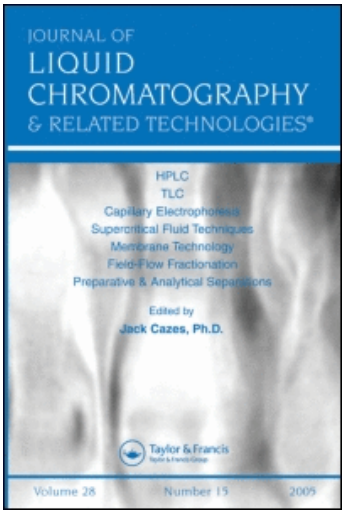

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ANALYSIS OF ORGANOPHOSPHORUS PESTICIDES IN VEGETABLE SAMPLES BY HOLLOW FIBER LIQUID PHASE MICROEXTRACTION COUPLED WITH GAS CHROMATOGRAPHY-ELECTRON CAPTURE DETECTION

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ANALYSIS OF ORGANOPHOSPHORUS PESTICIDES IN VEGETABLE SAMPLES BY HOLLOW FIBER LIQUID PHASE MICROEXTRACTION COUPLED WITH GAS CHROMATOGRAPHY-ELECTRON CAPTURE DETECTION

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□ *A method based on hollow fibre liquid phase microextraction (HF-LPME) coupled with gas chromatography electron capture detection (GC-ECD) has been developed for the determination of organophosphorus pesticides (OPPs) (chlorpyrifos and profenofos) in vegetable samples. In this method, a microsyringe needle with 1.5 cm polypropylene hollow fibre containing a volume of organic acceptor phase (n-dodecane) was immersed in an aqueous donor solution, and at the completion of extraction, the acceptor phase was withdrawn and transferred to GC-ECD for analysis. The effects of extraction solvent, volume of acceptor phase, and volume of donor phase were investigated. The optimized conditions for HF-LPME of the selected OPPs were n-dodecane as organic solvent, 11 mL of donor phase, and 3 μL of acceptor phase. The correlation coefficient (r^2) of the calibration curves ranged from 0.998 to 0.999. The limits of detection (LOD) were between 0.099 and 0.128 μg/mL. The developed method provided excellent RSDs ranging from 0.54% to 8.00% and analyte recoveries ranging from 60.8% to 88.0%. This method was applied successfully for determination of organophosphorus pesticides in selected vegetables.*

Keywords GC-ECD, hollow-fiber liquid phase microextraction, organophosphorus pesticides

INTRODUCTION

According to the status list of all active pesticide substances on the European Union (EU) market, more than 1100 pesticides are currently

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registered.[1] The pesticides industry is made up of companies, both multinational and local companies that are involved in manufacturing, formulating, or trading activities. The majority of pesticides are imported as technical materials, which are then blended, diluted, or formulated. Pesticides are widely used for agricultural activities due to their relatively low price and high effective ability to control pests, weeds, and diseases.[2] The increasing production ofpesticides for agricultural and non-agricultural purposes has caused the pollution of air, soil, ground, and surface water, which involves a serious risk to the environment, as well as human health, due to either direct exposure or through residues in food and drinking water.[3] The need for accurate determination of pesticides at the trace levels in the environmental samples is therefore obvious. With the improvement of self safeguard consciousness and the development in analytical instruments, levels of pesticides in vegetables and fruits are currently regulated by international and national organizations and maximum residue levels (MRLs) have been established in many countries.[4]

In this study, two organophosphorus pesticides (OPPs) were chosen namely, chlorpyrifos and profenofos. Chlorpyrifos is an organophosphate insecticide originally used primarily to kill mosquitoes but it is no longer registered for this use. Cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, and lice can be controlled effectively by chlorpyrifos. It is also used as an insecticide on grain, fruit, nut, and vegetable crops, as well as on lawns and ornamental plants. It is also registered for direct use on sheep and turkeys, for horse site treatment, dog kennels, domestic dwellings, farm buildings, storage bins, and also commercial establishments. Meanwhile, profenofos is used to control tobacco budworm, cotton bollworm, armyworms (fall, beet), cotton aphid, spider mites, plant bugs, flea hoppers, and white fliers. Repeated or prolonged exposure to organophosphate may result in impaired memory and concentration, disorientation, severe depression, irritability, confusion, headache, speech difficulties, delayed reaction times, nightmares, sleepwalking, and insomnia.[5]

Sample preparation is normally required to isolate and concentrate compounds of interest from the sample matrix, before analysis.[6] Ultimately, the concentration of target compounds is enhanced (enrichment) and the presence of matrix components is reduced (sample clean up). In order to achieve a low detection limit, an enrichment step should be conducted prior to analysis.[7]

Liquid–liquid extraction (LLE) and solid phase extraction (SPE) are the classical techniques for sample preconcentration and isolation in chemical analysis. However, LLE and SPE are time consuming, generally labour intensive, and requires use of large amounts of expensive high

purity organic solvents, which are often hazardous. During the last 10 years, some interest has been focused on the miniaturization of analytical LLE. The major idea behind this has been to facilitate automation, to speed up extractions, and to reduce the consumption of organic solvents.

An attractive alternative pretreatment method to the traditional technique is solid phase microextraction (SPME). SPME was developed by Pawliszyn and co workers.[8] SPME is a solvent free extraction technique that incorporates sample pretreatment, concentration, and sample introduction into a single procedure.[9] However the extraction fiber is expensive, fragile, and has a limited lifetime.[10,11] Miniaturized LLE or liquid phase microextraction (LPME), was first introduced in 1996 by Jeannot and Cantwell,[12] and was based on a droplet of organic solvent hanging at the end of a micro syringe needle. Although hanging drop LPME is very simple and efficient, and reduces the consumption of organic solvents per sample to a few μL, it is still used only in a limited number of research laboratories. One reason for this may be the low stability of the hanging drop, which is easily lost into the sample during extraction.

Alternatively, miniaturized LLE may be accomplished by hollow fiber protected LPME (HF-LPME). In this system, a small volume of extracting liquid is contained within the lumen of a porous hollow fiber. The major advantage of this is that the extracting liquid is mechanically protected, and it is prevented from leaking into the sample during extraction. This is especially important since LPME is conducted with strong agitation of the sample to speed up the extractions. Hollow fiber LPME can be accomplished both in the two and three phase modes. Furthermore, this technique can give a high degree of analyte selectivity and enrichment and eliminating the possibility of carry over between runs.

Because of these advantages, the HF-LPME technique has been applied in the analysis of various samples including pesticides. Pan and Ho reported the use of (HF-LPME) coupled with gas chromatographic electron capture detection (GC-ECD) for the determination of six fungicides (chlorothalonil, hexaconazole, penconazole, procymidone, tetraconazole, and vinclozolin) in water samples.[3] Huang and Huang studied the potential of dynamic HF-LPME for the extraction of organochlorine pesticides (OCPs) in green tea leaves and in commercially sold ready to drink tea prior to GC-ECD analysis,[13] while Lambropoulou and Albanis demonstrated that a selective trace enrichment of environmental andiandrogen vinclozolin from natural water samples can be achieved by the HF-LPME method.[14]

The objectives of this study are to determine the optimum conditions for the analysis of OPPs in HF-LPME and to investigate the appplication

of HF-LPME in the determination of OPPs in vegetable samples. Three important parameters are studied in this work: selection of organic solvent, volume of acceptor phase, and volume of donor phase. The selection criteria for a suitable organic solvent are: i), it should be easily immobilized in the pores of the polypropylene hollow fiber, ii), the organic solvent should be of low volatility to avoid solvent loss and iii), it must be immiscible with water. The sensitivity of the method can be increased by decreasing the volume ratio of the acceptor to donor phase. It is unnecessary to use a large volume of organic solvent (acceptor volume), since the typical injection volume is 1 μL. Separations and quantification of the analytes were performed by GC-ECD.

EXPERIMENTAL

Chemicals and Reagents

Chlorpyrifos was from Sigma-Aldrich (USA) while profenofos and vinclozolin (internal standard) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Acetonitrile (HPLC solvent, 99.9%) was obtained from J. T. Baker (USA). Stock solution (1000 μg/mL) of each pesticide was prepared in 10 mL volumetric flask with acetonitrile as the solvent. Working standard solutions were prepared by diluting the stock solutions with acetonitrile. The stock solutions and working standard solutions were stored in the freezer at 4°C. Deionized water of at least 18 MΩ was purified by the Nano ultra pure water system (Barnstead, USA).

Analytical Instrumentation

A 1 μL aliquot of the extract was injected into the Perkin Elmer Autosystem XL gas chromatograph equipped with an ECD detector (San Jose, United States). The capillary column used was an Ultra 2 column, 25 m × 0.2 mm i.d. and 0.33 μm film thicknesses. Helium gas was used as the carrier gas with flow rate of 1.1 mL min^{-1} and nitrogen was used as the make up gas with flow rate of 32.4 mL min^{-1}. The data were interpreted using Perkin Elmer software Turbochrom Navigator version 4.1.

GC-ECD Operating Conditions

For desorption of OPPs inside the GC injection port, the temperature employed was 260°C. The column (Ultra 2) was kept at 200°C for 2 min, and then ramped at 4°C min^{-1} to 220°C, held for 1 min, and finally ramped at 10°C min^{-1} to 290°C, and held for 1 min. The detector was set at 300°C.

Helium was used as the carrier gas and nitrogen as the make up gas. Peak area ratio of OPPs to vinclozolin in the ECD chromatogram signals were used to demonstrate the effect of parameters on the extraction and the efficiency of the extraction.

Liquid Phase Microextraction

Accurel Q3/2 polypropylene hollow fiber membrane (600 μm i.d., 200 μm wall thickness, and 0.2 μm pore size) was purchased from Membrane (Wuppertal, Germany). A 10 μL Hamilton syringe (Switzerland) with a cone tip was used to introduce the acceptor phase into the polypropylene hollow fiber membrane. The hollow fiber was cut manually to short pieces of 1.5 cm. In the optimized method, the hollow fiber was cleaned ultrasonically for 5 min in acetone to remove contaminants before use. The solvent was allowed to evaporate completely. For each extraction, a new piece of hollow fiber was used to prevent the carry over effect. The polypropylene hollow fiber membrane was sealed on one edge by a sealer machine, and then immersed into the acceptor phase (e.g., *n*-dodecane) for 10 sec to impregnate the pores of the fiber with the acceptor phase. The needle of the microsyringe that contained the acceptor phase (3 μL) was inserted through the septum of the sample vial. The microsyringe needle tip was then inserted into the hollow fiber segment and the assembly was immersed in the 11 mL sample solution. After that, the microsyringe was fixed on the retort stand. The sample was stirred using a magnetic stirrer during the extraction process. After a prescribed time, the solvent in the fibre was retracted into the syringe and injected into the GC-ECD for analysis.

Sample Preparation

Three types of vegetables chosen were tomato, cabbage, and water convolvulus. The samples were purchased from a supermarket in Taman Universiti, Skudai, Johor, Malaysia. They were rinsed slightly to remove adhering soil and stored in the refrigerator to keep them fresh before analysis. The vegetable samples were chopped and homogenized using a GNC blender (Panasonic, Malaysia). An aliquot (12 g) of the vegetables of the homogenized sample were weighed into a 50 mL centrifuge tube and 20 mL acetone was added. The samples were extracted for 30 minutes in an ultrasonic bath. Then, they are filtered through a Whatman filter paper. Samples were spiked with 2.5 μg/mL of standard OPPs mixture after confirming no OPPs were detected in the sample using GC-ECD.

RESULTS AND DISCUSSION

Optimization of Extraction Conditions

Selection of Extraction Solvent

The type of organic solvent immobilized in the pores of the hollow fiber is an essential consideration for efficient analyte preconcentration. As in LLE, the principle "like dissolves like" also applies to LPME. The solvent should be of low volatility to prevent evaporation, low viscosity to ensure rapid mass transfer, low polarity to ensure compatibility with the hollow fiber,[15] and to prevent leakage into the sample. Also, the solvent should provide high distribution constants for the target analytes.[16,17] High polarity solvents are not suitable for LPME due to solvent leakage via the hollow fiber pores.

Based on the preliminary experiments, three organic solvents namely acetonitrile, dichloromethane, and *n*-dodecane were evaluated. *n*-Dodecane is a non-polar solvent compared to dichloromethane. Meanwhile, acetonitrile is a polar solvent.

Figure 1 shows the influence of extraction solvents towards extraction efficiency of two OPPs. Based on the figure, *n*-dodecane extracted the analytes better than the other two solvents. This is due to the facts that the OPPs are non-polar, thus a non-polar solvent is required to extract OPPs successfully. *n*-Dodecane was thus utilized as the extraction solvent for the rest of the study.

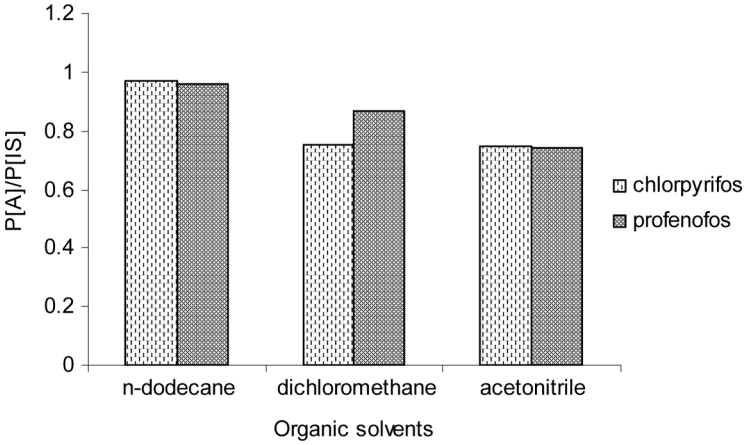

FIGURE 1 Effect of extraction solvent on HF-LPME. Conditions: extracting solvent volume: 3 µL, length of fiber: 1.5 cm, sample volume: 11 mL, stirring rate: 1360 rpm, extraction time: 30 min, concentration of chlorpyrifos: 10 µg/mL, concentration of profenofos: 10 µg/mL.

Effect of Volume of Donor Phase and Volume of Acceptor Phase

Two phase HF-LPME can give higher recoveries since the ratio of volume of donor phase over volume of acceptor is high. In this work, 1 µL to 3 µL of acceptor phase and different types of donor phase volume (10 mL, 11 mL, 12 mL) were evaluated. The peak areas of the analytes were found to vary significantly with donor phase and acceptor phase volume (Figure 2). From the extraction, 3 µL of acceptor phase and 11 mL of donor phase were utilized as the optimum volume throughout the experiments. The separation of the selected analytes at optimum conditions is shown in Figure 3.

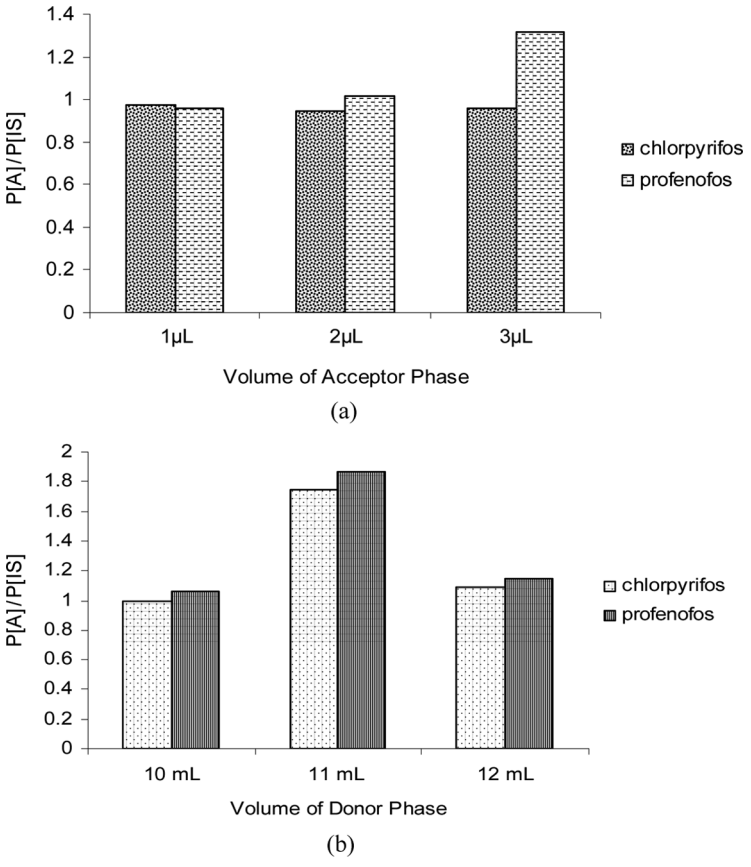

FIGURE 2 Effect of volume of (a) acceptor and (b) donor phase in the extraction. Extraction conditions: acceptor phase: *n*-dodecane, length of fibre: 1.5 cm, stirring rate: 1360 rpm, extraction time: 30 min, concentration of chlorpyrifos: 10 µg/mL, concentration of profenofos: 10 µg/mL.

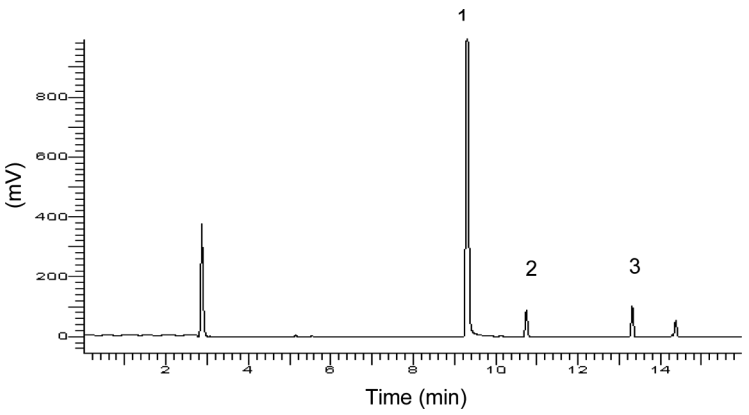

FIGURE 3 GC-ECD chromatogram of OPPs obtained by HF-LPME under optimized conditions. Peaks: (1) vinclozolin (internal standard) (2) chlorpyrifos, (3) profenofos.

Performance of HF-LPME Procedure

For validation of the HF-LPME method, optimized conditions (*n*-dodecane as extraction solvent, volume of acceptor phase: 3 μL, and volume of donor phase: 11 mL) were used for the extraction of OPPs using HF-LPME. The correlation coefficient, LOD and LOQ of each pesticide are shown in Table 1.

Limit of detection (LODs) were calculated as three times the standard deviation of three replicate runs of spiked samples at the lowest concentration of the analytes. The LODs of the pesticides were 0.12 μg/mL for chlorpyrifos, and 0.099 μg/mL for profenofos. They are well below the MRL set by Codex Alimentarius Commission (CAC), which is under 1 μg/mL in vegetable samples.

Real Sample Analysis

In order to investigate the applicability of the proposed trace enrichment microextraction method, three vegetable samples were studied. The optimum conditions obtained in the optimization of two phase HF-LPME was then applied to the determination of pesticides in the selected vegetable samples. No pesticide residues were detected in the samples. To demonstrate the applicability of the method for vegetable samples analysis, (cabbage, tomato, and water convolvulus) samples were spiked with the

TABLE 1 Correlation Coefficients, LOD and LOQ of OPPs Studied Using HF-LPME Coupled with GC-ECD

Pesticides	Correlation Coefficients, r^2	LOD (μg/mL)	LOQ (μg/mL)
Chlorpyrifos	0.998	0.128	0.427
Profenofos	0.998	0.099	0.331

studied analytes. The vegetable samples were spiked with 2.5 μg/mL pesticides and analyzed in triplicate by the proposed method and the representative chromatograms can be seen in Figure 4 (a, b, and c, respectively).

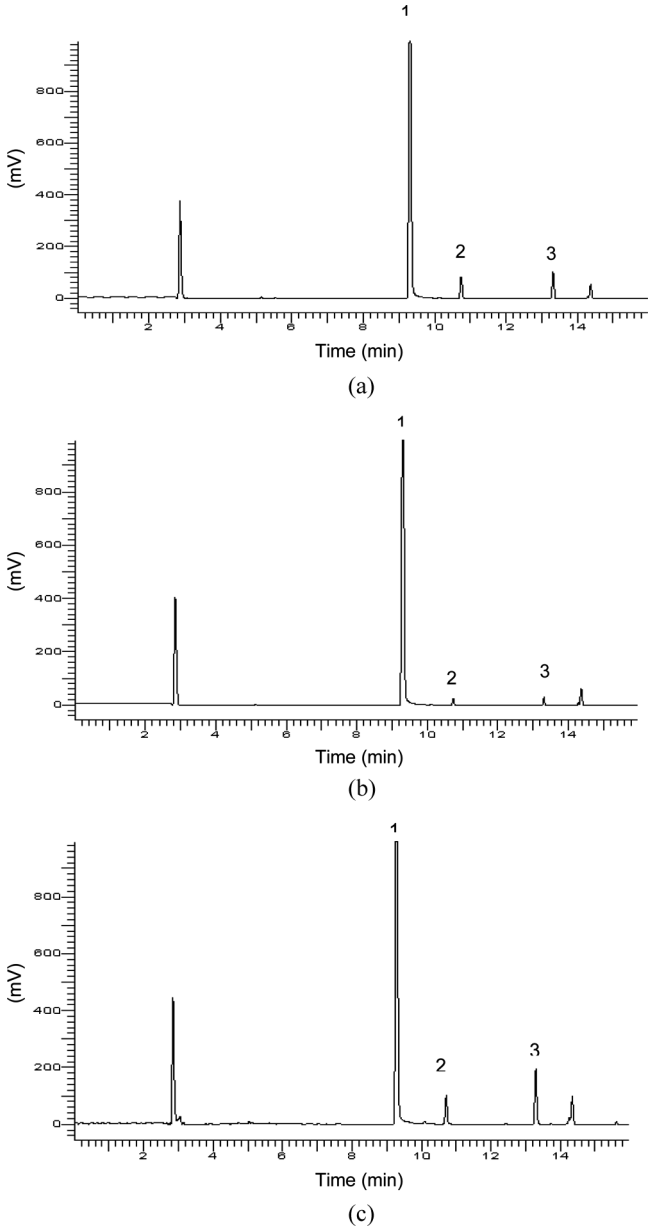

FIGURE 4 GC-ECD chromatogram of OPPs obtained by HF-LPME under optimized conditions (a) spiked cabbage (b) spiked tomato (c) spiked water convolvulus. Peaks: (1) vinclozolin (internal standard) (2) chlorpyrifos, (3) profenofos.

TABLE 2 Recoveries and Relative Standard Deviations of OPPs in Real Samples

Recoveries of Real Samples (%) and Relative Standard Deviation (%)			
Analytes	Cabbage	Tomato	Water Convolvulus
Chlorpyrifos	80.4 ± 8.00	60.8 ± 3.52	80.8 ± 0.54
Profenofos	84.0 ± 3.36	64.8 ± 0.54	88.0 ± 0.43

The recoveries were calculated for triplicate samples. Table 2 shows the relative recoveries of the spiked samples, respectively. In general, the relative recovery ranged between 60.8% to 88% with RSD less than 10%.

In the spiked technique, high recoveries in the analysis of the samples usually correspond to high accuracy. The relative recovery is calculated from percentage of standard pesticides spiked expected area by comparison with standard solution of pesticides at the same level. More than 80% relative recoveries were obtained for most of the analytes in two of the three samples namely cabbage and water convolvulus samples, indicating that there was only a minor influence of sample matrix on the extraction. However, the recoveries for tomato were 60% for chlorpyrifos and 64.8% for profenofos. The matrix effect on the LPME extraction is probably attributed to the selectivity of the hollow fiber because of the pores in its wall, which act as a filter in the "dirty" sample, since large molecules, which can also be soluble in the organic solvent, will not be extracted.

CONCLUSIONS

A method based on HF-LPME coupled with GC-ECD was developed for the determination of OPPs in vegetable samples. The limit of detection (LOD) was between 99 μg/L and 128 μg/L while limit of quantification (LOQ) was between 331 μg/L and 427 μg/L. The recovery was in the range 60–88%. The relative standard deviations were in the range of 0.54–8%. The method has the merits of simplicity, low cost, and relatively short time for equilibrium extraction.

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