

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF PESTICIDE RESIDUES IN THE BLOOD AND RESULTANT HEALTH ISSUES IN THE PEOPLE OF KARACHI - PAKISTAN

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ABSTRACT

The present study aimed to quantify the status of pesticide residues in the blood sample of persons representing from different regions of Karachi, Pakistan. Total 45 persons exposed to pesticides were selected in the study for high performance liquid chromatographic quantification of residues. After analysis of all the blood samples, the results indicated that only one sample contains malathion, two samples showed the presence of polyrin-C while Cypermethrin and Deltamethrin were found in almost all the samples. Rest of the Pesticides such as DDE, DDT, diazinon and monocrotophos were not found in any sample.

Keywords: Pesticide, Residues, Blood, Health issues.

INTRODUCTION

Pesticides comprise of various chemicals that are deliberately inducted in the environment for the purpose of eliminating different pests. The main consumption of pesticides is in the agricultural sector. As pesticides are basically pollutants or mixture of poisonous substances that are found in our environment can be beneficial in eliminating or killing undesirable organisms but as they are toxic in nature so their application or use in the field or home should be controlled because their misuse can cause serious disturbances to human health. (Azmi and Naqvi, 2011).

In the agriculture sector most of the persons from Pakistan consider commercial-based pesticides for the protection of their agricultural products from the attack of harmful toxic organisms but these commercial or traditional pesticides causing harm to the people and to the environment as well. Based on environmental contamination the developing countries replaced these commercial pesticides with plant-based pesticides as they are not only effective for the field products but also safe to the environment and human health.

It is important to mention that there are number of important factors that can cause contamination or poisoning among farm workers in the developing countries like frequent use of pesticides by the persons or through dermal, oral, eye and inhalation routes of contact (Wesseling *et al.*, 2001; Konradson *et al.*, 2003, Coronado *et al.*, 2004). A number of studies in this connection have proved that misuse or mishandling of pesticides creates serious disturbances on human health and environment. (Soares *et al.*, 2003; Mancini *et al.*, 2005; Remor *et al.*, 2009).

Extensive research has been done in this connection and reported the detection of pesticides such as polychlorinated biphenyls and organochlorine pesticides in water and human blood through several techniques. They are potentially dangerous for human health (Kocan *et al.*, 1994; Ntow, 2001; Charlier and Plomteux 2002, Butler *et al.*, 2003, Van Oostadam., 2004; Lopez *et al.*, 2004).

Polychlorinated biphenyls and some common organochlorines have been reported from the sediments and coastal regions of Egypt and in the serum samples of people from Slovak. (Barkat *et al.*, 2013; Jana *et al.*, 2014). Similarly, Sharma *et al.*, (2015) monitored the status of pesticide residues of persons from India. Related to the pesticide exposure and health issues, Khan and Samalas (2015) also observed the same findings in the farmers of Punjab from Pakistan. In such concern Sharama *et al.*, (2016) reported the use of spectroscopic methods and Fourier transform infrared coupled with solid phase extraction method for the detection of malathion level in the blood plasma of human.

Keeping the hazardous effects in priority, present study has been under taken to determine the quantity of pesticides in the blood as well as to assess their harmful effects in persons belonging from different socio-economic regions of Karachi-Pakistan.

MATERIALS AND METHODS

Collection of blood Samples

Blood samples were collected from patients of Fatimid Foundation belonging from different regions of Karachi were selected. The blood samples of all persons were collected in well-cleaned heparinized glass tubes and saved in

ice box during collection. The samples were immediately brought to the laboratory for the centrifugation. Serum were separated out and transferred into glass vials. All the vials containing serum samples were placed in freezer for analysis.

Quantification of pesticide residues

Quantification of residues of pesticides in the blood sample was carried out through high performance liquid chromatography (HPLC) which is attached with electron capture detector. The different steps involved for quantification are: Fat extraction method (Kadoum, 1967), Soxhlation method (Holden and Marsden, 1969) and Sorption process consisting of alumina chromatographic column method (Holden and Marsden, 1969) and Silica chromatographic column (Kadoum, 1967; 1968) method. All the parameters required for quantification process were set on HPLC Shimadzu SPD-10A apparatus attached with chart recorder. The standard pesticides and blood samples were prepared and processed according to Dale *et al.*, (1940) method. After processing ten (10) μL of each sample was injected into the HPLC apparatus through special chromatographic syringe for obtaining respective chromatograms. Quantification was then done by comparing the peaks of sample chromatogram with the standard peaks on the basis of retention time. The area of each sample peak was calculated to quantify the residues in the blood sample.

RESULTS AND DISCUSSION

All the blood samples after centrifugation and clean up procedures were brought to HPLC for residual analysis and were found to have sufficient quantity of pesticide residues (**Table 1**). In the present study 45 persons in total from different locations of Karachi were selected to find out the different pesticides in their blood. Out of these only 20 persons showed severe exposure to pesticides and were considered for HPLC analysis. Ten different standard pesticides were taken into consideration in this study and only five pesticides were detected in exposed persons. The pesticides that were detected included Cypermethrin, Deltamethrin, polytrin-C, malathion and permethrin (**Table 2; chromatogram I-V**). The retention time (RT) of all the standards is given in **Table 2**. In addition 10 normal control persons were also included in the study for the quantification of pesticide residues.

It has been found that out of 20 samples, Cypermethrin was detected in 10 samples. Low quantity was found in 4 samples and the lowest $0.32 \mu\text{g}/\text{mL}$ was noted in **sample no. 32** in male person named **Sarfraz** (32 yrs). It was also found in higher quantity in 6 samples and the highest was found in female person in **sample no. 42** named **Nusrat** (32 yrs) i.e., $26.8 \mu\text{g}/\text{mL}$ (**chromatogram-Ia**). The high quantity in this subject may be due to longer exposure. In rest of the samples Cypemethrin was not detected.

Deltamethrin was noted in the blood samples of 13 persons and the highest quantity was in **samples no. 33** i.e., $34.86 \mu\text{g}/\text{mL}$ (**chromatogram-IIa**) named **M. Yousuf** (M) aged 29 years possibly because of direct exposure or use of Deltamethrin frequently by this person. Similar finding is also reported by Jahan (1995) i.e., $12.22 \mu\text{g}/\text{mL}$ and Bissacot and Vassilieff (1997). Low quantity of Deltamethrin was noted in the blood of 7 persons where as in other persons Deltamethrin was not detected in a detectable quantity.

A high amount of polytrin-C i.e., $16.15 \mu\text{g}/\text{mL}$ was found in male person named **Shehzad** in **sample no.19** and a very high quantity $30.76 \mu\text{g}/\text{mL}$ (**chromatogram-IIIa**) was noticed in a very young male subject in **sample no. 27**. This high quantity may be due to the greater exposure or improper use. No other worker showed detectable quantity in any of the samples.

Malathion was detected in high quantity i.e., $12.6 \mu\text{g}/\text{mL}$ (**chromatogram-IVa**) in the person named **Ali Mohammad** aged 33 years in **sample no. 41**. No detectable quantity of malathion was seen in rest of the samples. Detection of malathion in the samples of human blood was also reported by many researchers (Abu-Qare and Abu-Donia, 2001; Musshoff *et al.*, 2002; Jaiswal *et al.*, 2008; Venugopal *et al.*, 2013).

Permethrin was detected in high quantity in 6 samples and the highest quantity $8.82 \mu\text{g}/\text{mL}$ (**chromatogram -Va**) was found in the blood sample of very young male person named **Jan Sher** from **sample no.4** out of total 6 samples. No low quantity was detected in any sample. Permethrin level in the blood sample was also reported by many researchers (Abu-Qare and Abu-Donia 2001; Lestremau *et al.*, 2014) in the blood samples of human and rat model.

Exposure to pesticides and resultant health problems in the highly exposed persons were also assessed indicated that their health being affected by the pesticide residues e.g., persons that were highly exposed with Cypermethrin in sufficient quantity were **Nusrat** $26.8 \mu\text{g}/\text{mL}$ (**sample no. 42**), **Asif** $17.31 \mu\text{g}/\text{mL}$ (**sample no.43**) and **M. Yousuf** $13.93 \mu\text{g}/\text{mL}$ (**sample no.33**). These persons complained about liver disorder and therefore suffered with hepatitis repeatedly. They might be highly affected due to the frequent exposure of this pesticide. In addition to hepatitis these people also complained about the backache and headache frequently while **M. Khan** complained about the burning sensation during micturition and hematuria. Some researchers also reported the health issues that are

produced due to the frequent exposure of Deltamethrin e.g., **Abdul Rahman (sample no. 29)** and **M. Yousuf (sample no. 33)** had high quantity of Deltamethrin i.e., 22.42 μ g/mL and 34.86 μ g/mL respectively in their blood. These people complained about the history of respiratory illness, altered lung functions and disturbed some of the hematological profiles. These findings are almost in line with the findings of some researchers (Goswamy *et al.*, 1994; Fareed *et al.*, 2013). **Shehzad** from (sample no.19) and **Aamir (sample no. 27)** with high values of profenophos 16.15 μ g/mL and 30.76 μ g/mL suffered with dermatitis, irritation and redness of eye, headache and dizziness during examination. The same findings were also noted by Mehboob *et al.*, 2012; Khan and Damalas, 2015).

Table 1. Pesticide residues in the blood samples of 20 persons from Karachi.

Blood Samples	Name Age (Sex)	Pesticide Residues (μ g/mL)					
		Cypermethrin	Deltamethrin	Polytrin-C		Malathion	Permethrin
				Cypermethrin	Profenophos		
Sample # 4	Marium 31 yrs (F)	-	1.71	-	-	-	-
Sample # 7	Akhter Hussain 26 yrs (M)	0.42	0.97	-	-	-	-
Sample # 8	Asim 30 yrs (M)	\uparrow 1.34	\uparrow 3.84	-	-	-	-
Sample # 10	Zeeshan 35 yrs (M)	-	-	-	-	-	\uparrow 3.58
Sample # 15	Ghulam Mustafa 28 yrs (M)	\uparrow 4.31	-	-	-	-	-
Sample # 19	Shehzad 31 yrs (M)	0.51	0.36	-	\uparrow 16.15	-	\uparrow 8.10
Sample # 20	Jan Sher 23 yrs (M)	-	-	-	-	-	\uparrow 8.82
Sample # 22	Fazal Ghani 45 yrs (M)	-	0.34	-	-	-	\uparrow 6.66
Sample # 24	Younus 30 yrs (M)	0.76	\uparrow 3.52	-	-	-	-
Sample # 25	M. Khan 35 yrs (M)	\uparrow 9.36	-	-	-	-	-
Sample # 27	Aamir 22 yrs (M)	-	0.34	-	\uparrow 30.76	-	\uparrow 6.15
Blood Samples	Name Age (Sex)	Pesticide Residues (μ g/mL)					
		Cypermethrin	Deltamethrin	Polytrin-C		Malathion	Permethrin
				Cypermethrin	Profenophos		
Sample # 29	Abdul Rahman 25 yrs (M)	-	\uparrow 22.42	-	-	-	\uparrow 4.0

Sample # 31	Imran Ahmed 27 yrs (M)	-	1.54	-	-	-	-
Sample # 32	Sarfraz 32 yrs (M)	0.38	↑4.64	-	-	-	-
Sample # 33	M. Yousuf 29 yrs (M)	↑13.93	↑34.86	-	-	-	-
Sample # 35	Irfan 30 yrs (M)	-	1.46	-	-	-	-
Sample # 37	Samad 26 yrs (M)	-	↑2.71	-	-	-	-
Sample # 41	Ali Muhamm ad 33 yrs (M)	-	-	-	-	↑12.6	-
Sample # 42	Nusrat 32 yrs (M)	↑26.8	-	-	-	-	-
Sample # 43	Asif 29 yrs (M)	↑17.31	-	-	-	-	-

Under line = Low value , ↑= High value, - = Not detected,

Table 2. Different parameters of standard pesticides.

No.	Pesticides	Retention Time (RT)	Concentration (%)	Quantity (µg/10µL)
1.	Match	3.243	0.019	1.9
2.	Cypermethrin	3.662	0.009	0.9
3.	Deltamethrin	2.638	0.016	1.6
4.	Polytrin- C • Cypermethrin • Profenophos	2.532	0.014	1.4
		4.192	0.014	1.4
5.	Diazinon	5.572	0.012	1.2
6.	DDE	5.297	0.014	1.4
7.	Monocrotophos	4.15	0.012	1.2
8.	Malathion	3.223	0.009	0.9
9.	DDT	6.153	0.006	0.6
10.	Permethrin	2.658	0.008	0.8

Flow rate = 1.0 mL/min, Absorbance = 0.02nm, Chart speed = 0.5 mm/min, Pressure = 200kg/mm, Wave length = 250nm, Sample injected = 10µL

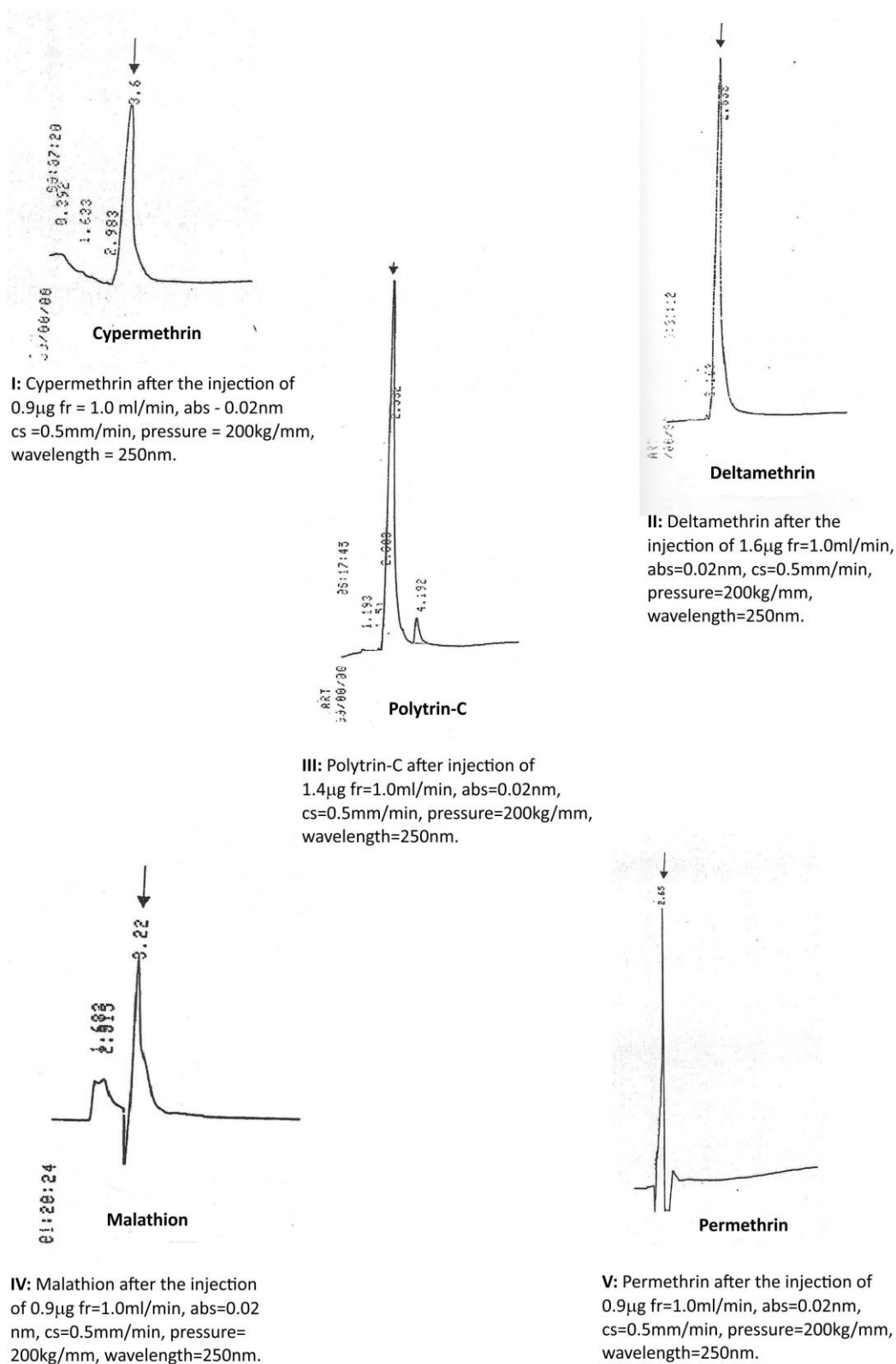


Fig.1. HPLC Chromatograms Showing the arrowed peaks (\downarrow) of standard pesticides Cypermethrin, Deltamethrin, polytrin-C malathion and permethrin.

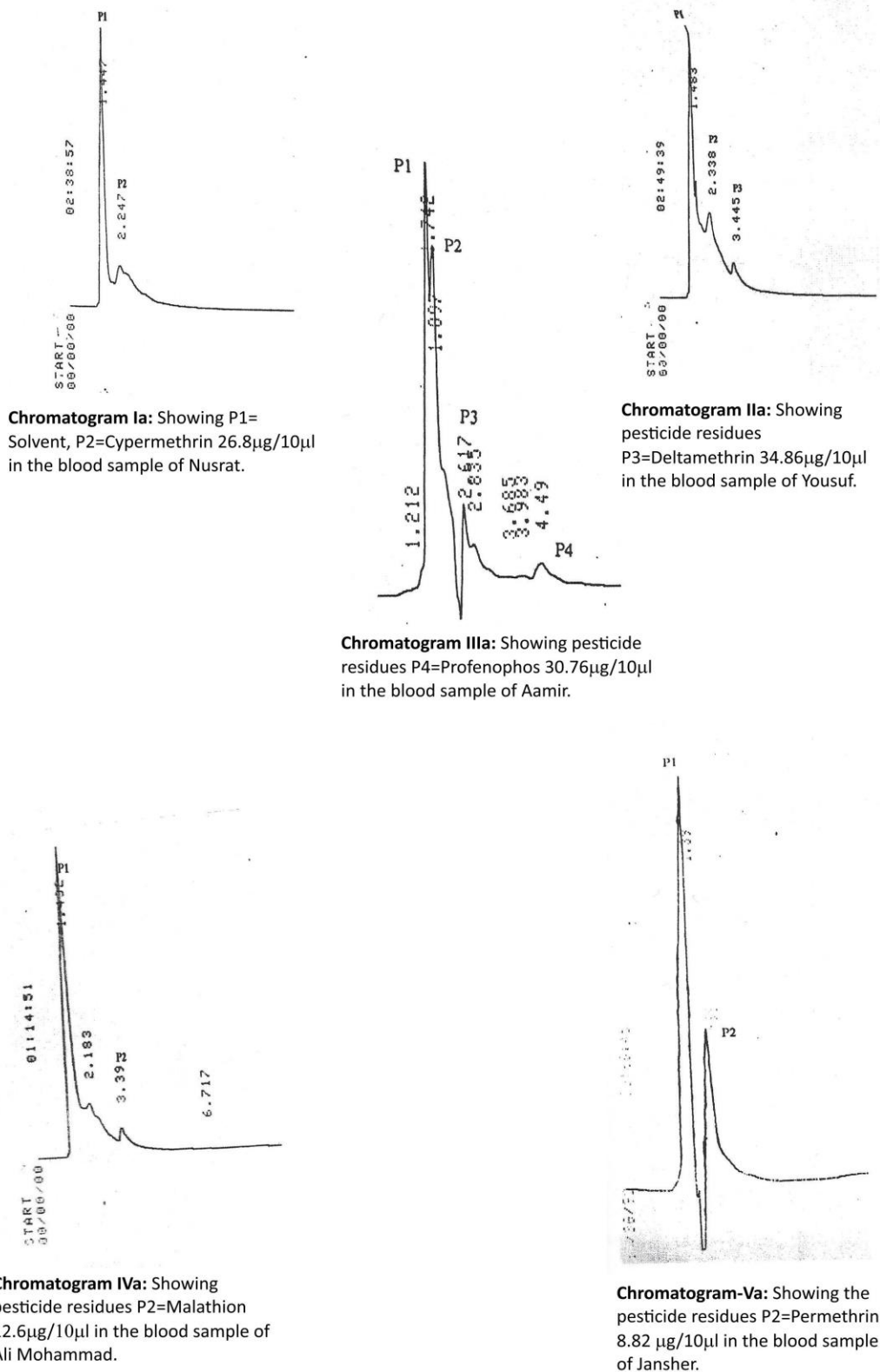


Fig. 2. HPLC chromatograms showing high quantity of different pesticide residues in the blood samples of highly exposed persons from different regions of Karachi.

Regarding malathion high quantity 12.6µg/mL was seen only in one person named **Ali Mohammad** of **sample no. 41**. In addition it is also noted that based on the level of exposure this person complained the regular sign and symptoms on examination of numbness, in hands backache, irritation of eye, lethargy, shortness of breath, in breathing weakness and on examination. (Choudhary *et al.*, 2008; Venugopal *et al.*, 2013; Moreira *et al.*, 2015). Similarly, **Jan Sher** of **sample no. 20** with permethrin 8.82 µg/mL remained sick throughout the year and complaining the frequent gastric pain and difficulty in breathing. The hazardous effects and health deterioration has also been reported by (Lestremau *et al.*, 2016).

It is thus concluded that on the basis of experimental data obtained it has been observed that Cypermethrin and Deltamethrin were present in sufficient quantity in some persons. Likewise polytrin-C, and permethrin also showed enough quantity but malathion was seen only in one person and other such as match, diazinon, DDT and monocrotophos were not found in any sample. So, it is clear that most of the detected pesticides were found in higher level than the maximum residual limit. Therefore, on the basis of experimental data and health issues it is suggested that as pesticides are basically poisonous in nature and the persons handling any pesticides must adopt safety measures by using gloves, spectacles and masks to protect them from the hazardous effects of pesticides.

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