

Extraction and Determination of Oxymatrine Pesticide in Environmental Sample and in its Formulation using High-Performance Liquid Chromatography

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Abstract—The quinolizidine alkaloid compound, oxymatrine pesticide, was analyzed in the river water samples collected from different agriculture areas in the Iraqi city of Kerbala and also in its formulation using developed reverse-phase high-performance liquid chromatography method. Acetonitrile:methanol (60:40 v/v) was chosen as mobile phase at pH (7.0), flow rate 0.5 mL/min, and 20 μ L as volume injection. Modified ecological-friendly method, dispersive liquid-liquid microextraction, was used for the extraction of oxymatrine from water samples. Linearity study was constructed from 0.1 to 70 μ g/mL at λ_{\max} 205 nm. The limit of detection and limit of quantification were 0.025 and 0.082 μ g/mL, respectively, and the relative standard deviation (RSD) % was 0.518%. Three spiked levels of concentration (20.0, 40.0, and 70.0 μ g/mL) were used for the validation method. The percentage recovery for the three spiked samples was ranged between 98.743 and 99.432 and the RSD% was between 0.051 and 0.202%, the formulation studies of oxymatrine between 99.487 and 99.798, and the RSD% was ranged from 0.045 to 0.057%. The developed method can be used accurately and selectively for the determination of oxymatrine in environmental samples and in the formulation.

Index Terms—Dispersive, Oxymatrine, Reverse-phase high-performance liquid chromatography, UV visible.

I. INTRODUCTION

Alkaloids are compounds which have low molecular weight which consist of nitrogen atom in its structure. Quinolizidine alkaloids are a type which can be found in 20% of plants. These quinolizidine alkaloids play an important role as pesticides and in biological activity as starting material

instead of acetylcholine to treat senile dementia due to its binding to cholinergic receptors called nicotinic and muscarinic receptors (John, et al., 2014).

Oxymatrine, tetracycloquinolizidine (7aS,13aR,13bR,13cS) dodecahydro-1H,5H,10H-dipyrido[2,1-f:3',2',1'-ij][1,6]naphthyridin-10-one 4-oxide, as shown in Fig. 1, is a new extracted insecticide from the roots of *Sophora flavescens* Aiton which used as Chinese herbal to treat cooling and as an antichloristic (Li and Wang, 2004). Oxymatrine regarded as a new biopesticide, under the name Levo2.4, instead of chemical pesticides to overcome the aggregation of residual in environment (Gu, et al., 2012; Zhang, et al., 2015a; Gholam and Sadeghi, 2016). Patient who suffers from hepatitis B can suffer from liver damage when they exposed to high dose of oxymatrine (Izdebska, et al., 2019).

Many techniques were reported for the analysis and determination of oxymatrine in plant as residual and in pharmaceutical preparations. Several techniques were used for the determination of oxymatrine, some of these techniques are high-performance liquid chromatography (HPLC) (Izdebska, et al., 2019; Bao, et al., 2019; Zhang, et al., 2016), liquid chromatography–mass spectroscopy (Zhang, et al., 2008; Fan, et al., 2013; Sabatino, et al., 2015; Jong, et al., 2006), capillary electrophoresis (Chen, et al., 2009; Zhang and Chen, 2013), flow injection (Cheng, et al., 2004), and microwave-assisted aqueous two-phase extraction (Zhang, et al., 2015b).

The aim of this study is to provide accurate, selective, and rapid method to the determination of oxymatrine in water samples and in its formulations used in agriculture areas under study because there is no such study which was reported in those areas for the determination of oxymatrine in spite of using this pesticide widely and repeatedly in those areas.

II. MATERIALS AND METHODOLOGIES

A. Reagent and Solutions

Oxymatrine standard (purity >99%) was purchased from Dr. Ehrenstorfer GmbH Company. All solvents (methanol,



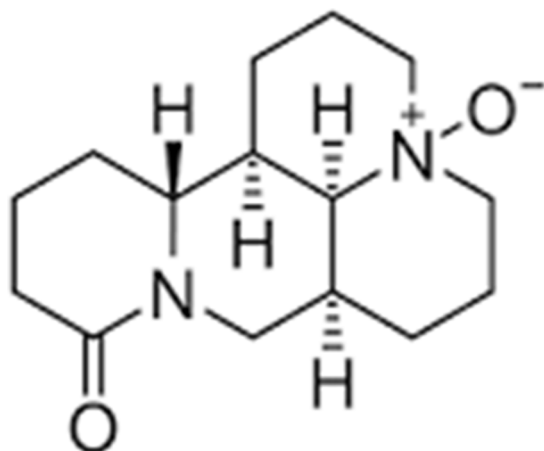


Fig. 1. Structure of oxymatrine.

acetonitrile, and water) for HPLC analysis were used from Sigma-Aldridge Company. Oxymatrine formulation (Levo2.4%) was supplied from Karbala Agriculture Department, Iraq.

B. Preparation of Standard Solutions

Oxymatrine stock standard solution 1000 $\mu\text{g/mL}$ was prepared by dissolving 0.1 g in methanol with sonication and completed to the mark in 100 mL volumetric flask, then filtered through 0.45 μm Millipore filter. A series of solutions ranged from 0.1 to 200 $\mu\text{g/mL}$ were prepared for calibration study. All solutions kept in 4°C until the time of analysis.

C. Preparation of Oxymatrine Formulation

A 1.0 mL of oxymatrine formulation (Levo 2.4%) was diluted to 100 mL, from this solution, further 1.0 mL was diluted to 100 mL, from the final solution, a different concentrations of formulation were prepared by diluting the calculated volumes of solution in 100 mL methanol.

D. Preparation of Sodium Hydroxide 0.1 M

Sodium hydroxide 0.1 M was prepared by dissolving 0.4 g in distilled water, then completed to 100 mL volumetric flask.

E. Preparation of Orthophosphoric Acid 0.1 M

Orthophosphoric acid was prepared by diluting 0.680 mL of concentrated orthophosphoric acid in distilled and completed the volume to 100 mL volumetric flask.

F. Preparation of Sodium Chloride (5%)

A 5.0 g of sodium chloride was dissolved in distilled water and completed to the mark in 100 mL volumetric flask.

III. SAMPLING

Water samples were collected from agriculture ears in Iraqi city – Kerbala at six different locations: Site I (Center of Kerbala), two agriculture eras were chosen (Al-Kadhy -8 and Al-Kamaliyah), site II (Al-Hindya), two locations chosen (South and North Al-Manfahan), and from site III (Al-

Khairat), two locations (Abu-Ruwayah and Zubaid) were collected chosen, then samples were transported in dark amber glass bottles to the laboratory under cooling conditions (4°C). All samples were filtered through 0.45 μm Nylon filter (Millipore filter) to remove any suspended particles or matrix in water samples, then the samples were stored at 4°C in the dark until the time of analysis.

IV. MODIFIED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION

A 5.0 mL from each water samples collected from different agriculture areas in Kerbala city, Iraq (reserved in standard conservation condition) were filtered and the supernatants were separated and transferred to a glass centrifuge test tube with screw. Each sample spiked with different concentration of oxymatrine standard (20.0, 40.0, and 60.0 $\mu\text{g/mL}$). Sodium chloride (5%) was added as salting-out agent, then a mixture of 100 μL chloroform, as dispersant agent, and 1.0 mL acetonitrile, as extraction solvent, were added, respectively. The final solution was vortexed for 1.0 min and further 10.0 min shaking by hand, then centrifuged at 5000 rpm for 15.0 min. Two phases will have separated; upper aqueous phase removed by microsyringe and organic phase contain extracted oxymatrine as fine droplets diluted with 2.0 mL methanol for HPLC analysis after filtration through 0.45 μm Millipore filter paper. Same procedure was maintained for water samples collected from different agriculture areas for Kerbala city, Iraq, without spiking to use as controller (Albaseera, et al., 2011).

V. INSTRUMENTATION

HPLC 20A equipped with UV–visible detector. Ultrasonic cleaner with heating controller, KQ200E. Shimadzu double-beam UV-spectrophotometer-1800. Digital balance, Denver-TP-214. pH meter, Hanna-pH211. Centrifuge, C2series.

VI. METHOD VALIDATION

A. Wavelength Selection

From the stock solution of standard oxymatrine, 20.0 $\mu\text{g/mL}$ solution was prepared for UV–visible scanning from 190 to 800 nm. It was found that standard oxymatrine has maximum absorption at 205 nm, Fig. 2.

B. Chromatographic Conditions

Analysis of oxymatrine in samples and formulation was applied using HPLC supplied with ODS-C18 column (250 cm \times 4.6 mm, 5 μm) and UV–visible detector. The mobile phase was acetonitrile:methanol (60:40 v/v) at pH (7). The flow rate was 0.5 mL/min and the volume of injection was 20.0 μL . The measurements were maintained at 205 nm.

VII. RESULTS AND DISCUSSION

A. Preliminary Study

Different composition of solvents was tested as mobile phase for the best separation, good resolution, and acceptable

peak shape. Acetonitrile, methanol, and water are used alone and as a mixture with composition from 90:10 v/v to 50:50 v/v to achieve an acceptable separation, Fig. 3. The mobile phase with the ratio (60:40 v/v) was chosen for optimization due to the best separation and high response. All investigations were being under flow rate 1 mL/min and volume injection 20.0 μ L at λ_{\max} 205 nm. Chromatogram for blank, Fig. 4, was explained for comparison with chromatogram of oxymatrine standard.

B. Effect of pH

Mobile phase was buffered with different pH to improve the peak shape and efficiency of alkaloid (oxymatrine) separation, for that, selected mobile phase (acetonitrile:methanol 60:40 v/v) was eluted under pH ranged from 3.0 to 8.0 using 0.1 M H_3PO_4 and/or 0.1 M NaOH for adjusting. Results obtained were illustrated in Table 1 and Fig. 5 for standard oxymatrine under optimized condition (pH = 7.0). In general, oxymatrine regarded as weak basic and its protonation can be altered in acidic and basic media, it was found that pH 7.0 was the best media for its separation.

Results in Table 1 and Fig. 5 explained that the separation of oxymatrine in acidic medium or high basic medium gave low intensity than in neutral medium depends on the properties of oxymatrine, for that pH (7.0) was chosen for optimization due to good resolution and acceptable values of capacity and tailing factors.

C. Effect of Flow Rate

A 20.0 μ L of 20.0 μ g/mL oxymatrine standard at pH (7.0) was injected to HPLC system (n=3) at various flow rates ranged from 0.3 to 1.5 mL/min.

TABLE I
EFFECT OF PH

pH	Peak area	Resolution (R)	Capacity (k)	Tailing factor (TF)
3.0	18,544	20.897	2.260	1.122
4.0	17,499	18.560	1.645	1.134
5.0	20,270	20.943	2.230	1.124
6.0	23,251	20.232	2.133	1.031
7.0	25,960	13.051	1.231	1.041
8.0	22,132	17.238	1.521	1.105

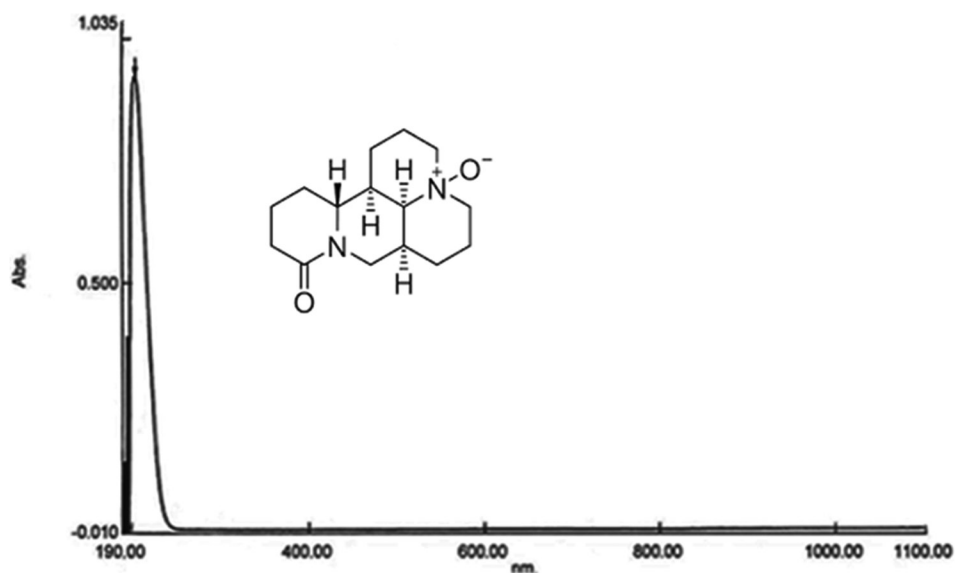


Fig. 2. UV-visible spectra for standard oxymatrine.

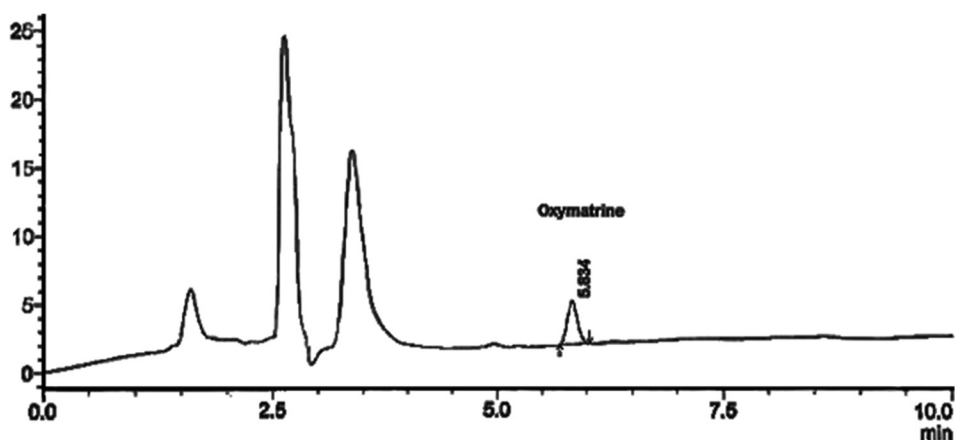


Fig. 3. Chromatogram of oxymatrine standard.

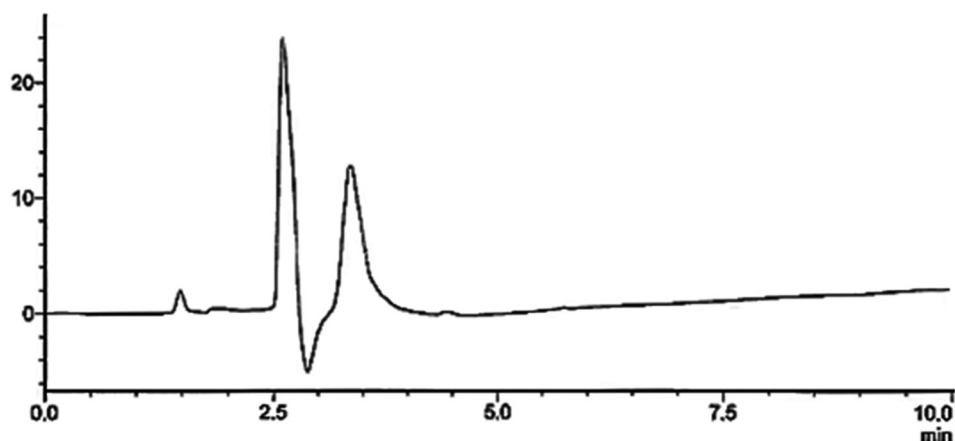


Fig. 4. Chromatogram of Blank.

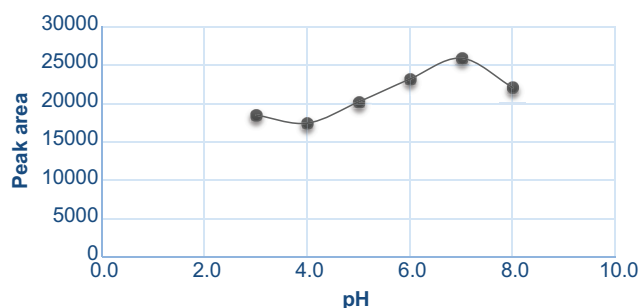


Fig. 5. Effect of pH.

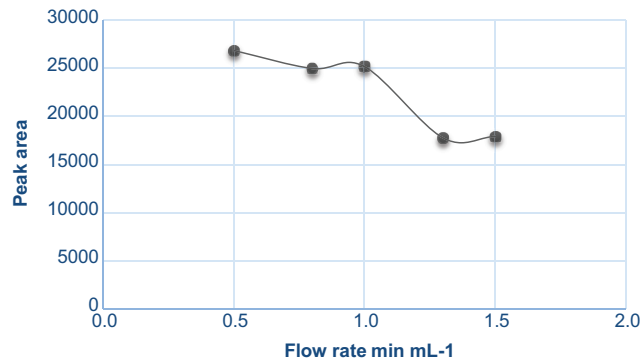


Fig. 6. Effect of flow rate.

Injection of oxymatrine standard into HPLC system at different flow rate explained that at 0.3 mL/min too long time for analysis was required (Table 2 and Fig. 6). Flow rate at 0.5 mL/min maintained high response with good capacity factor and low tailing factor after that the response decreases up to 1.5 mL/min. For that, 0.5 mL/min was chosen for oxymatrine determination.

D. Effect of Volume Injection

A 20.0 μL of 20.0 $\mu\text{g/mL}$ of oxymatrine standard under optimized conditions of pH and flow rate was injected ($n=3$) to achieve the best separation.

From results shown in Table 3 and Fig. 7, increasing the injected volume of oxymatrine standard up to 20.0 μL led

TABLE II
EFFECT OF FLOW RATE

Flow rate mL/min	Peak area	Resolution (R)	Capacity	Tailing factor (TF)
0.3		No detection till 20 min		
0.5	26,048	10.643	2.963	1.123
0.8	25,001	9.953	2.787	1.129
1.0	25,193	8.983	1.196	1.135
1.3	17,783	8.179	1.614	1.152
1.5	17,931	7.421	1.648	1.182

TABLE III
EFFECT OF VOLUME INJECTION

Volume injection (μL)	Area	Resolution (R)	Capacity (k)	Tailing factor (TF)
5	9502	9.117	3.061	1.100
10	15,348	9.043	3.008	1.132
15	20,771	8.681	2.993	1.119
20	26,855	10.643	2.963	1.023

TABLE IV
STATISTICAL DATA ANALYSIS OF DETERMINATION OF OXYMATRINE

Parameter	Results
λ max (nm)	205
Correlation coefficient	0.9999
Linearity range ($\mu\text{g/mL}$)	0.1–70
Limit of detection ^a ($\mu\text{g/mL}$)	0.024
Limit of quantification ^b ($\mu\text{g/mL}$)	0.082
Regression equation	$y=1219.6x+1853.1$
Slope	1219.6
Recovery %	100.002%
RSD%	0.518
C.L ^c for 0.5 $\mu\text{g/mL}$	0.498 ± 0.004
C.L for 10.0 $\mu\text{g/mL}$	10.134 ± 0.013
C.L for 50.0 $\mu\text{g/mL}$	49.757 ± 0.020

^aLOD=(SD/S) \times 3. ^bLOQ=(SD/S) \times 10. Where, SD is the standard deviation, S is the slope of calibration curve (Li, et al., 2016). ^cCL= $\bar{X}\pm t$ (SD/(n)^{1/2}). Where, \bar{X} is rate of measurement, t is the t-test at n-1 from degree of freedom at 95%, N number of sample (number of degree of freedom) (19). ^dRSD%=(SD/ \bar{X}) \times 100. LOD: Limit of detection, LOQ: Limit of quantification, SD: Confidence limit, RSD: Relative standard deviation

to increase the response systematically without overloaded of the column and without affecting the capacity factor of the shape of chromatogram.

E. Linearity Curve

A series of solution with different concentration ranged from 0.1 to 200 µg/mL were prepared for the calibration study at optimized conditions. The calibration curve obtained by plotting the peak area of injected solutions (n=3) against the concentration. Linearity was achieved in the range from 0.1 to 70 µg/mL, Fig. 8, after that increasing the concentration led to deviation from Beer's law. All results related to calibration curve study are sorted in Table 4.

VIII. VALIDATION METHOD

Validation of method was a proved in term of accuracy and precision. Accuracy was represented by recovery percentage

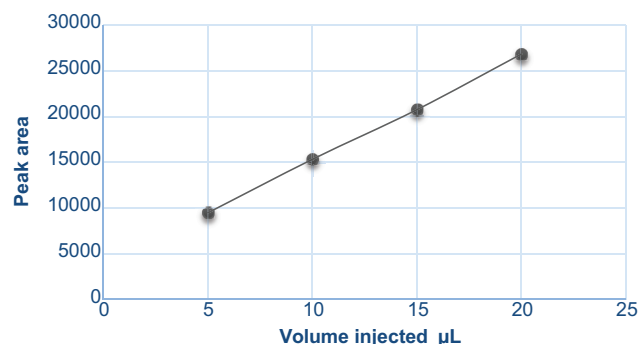


Fig. 7. Effect of volume injection.

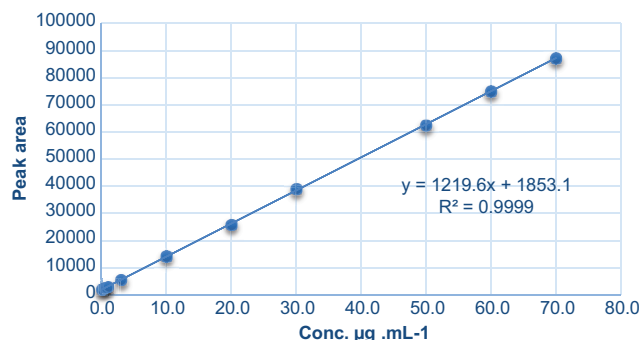


Fig. 8. Calibration curve of oxymatrine.

value and precision by relative standard deviation (RSD) %. Three different concentrations of prepared oxymatrine standards were injected (n=5) into HPLC system under optimized condition for validation studies. Results are illustrated in Table 5.

IX. APPLICATIONS

The proposed method was applied for the determination of oxymatrine in spiked water samples with different concentration and also its formulation. The results are shown in Tables 6, 7 and Figs. 9-13. Values of recoveries (98.745–99.432) and RSD% (0.202–0.064) obtained for the determination of oxymatrine in spiked water samples in addition to the acceptable values of recovery and RSD% for oxymatrine in its formulation approved that the method was precise and accurate.

Results in Tables 6, 7 and Figs. 9, 10 explained that the applied method was accurate and selective for the

TABLE V
ACCURACY AND PRECISION OF DETERMINATION OF OXYMATRINE STANDARS

Concentration (µg/mL)	Found (µg/mL) (n=5)	Recovery% (n=5)	RSD% (n=5)
0.5	0.465	93.096	0.698
10.0	10.109	101.078	0.107
50.0	49.752	99.505	0.033

TABLE VI
DETERMINATION OF OXYMATRINE IN SPIKED SAMPLES

Added (µg/mL)	Found (µg/mL) (n=5)	Recovery % (n=5)	RSD% (n=5)
20	19.748	98.745	0.202
40	39.772	99.432	0.051
70	69.236	98.908	0.064

TABLE VII
DETERMINATION OF OXYMATRINE IN FORMULATION

Added (µg/mL)	Found (µg/mL)	Recovery % (n=5)	RSD% (n=5)
30	29.846	99.487	0.057
60	59.879	99.798	0.045

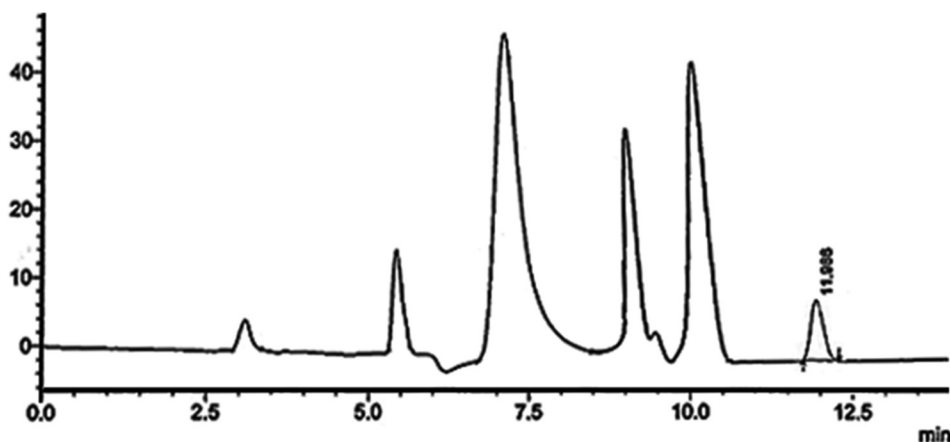


Fig. 9. Chromatograms for spiked water samples with 20.0 µg/mL oxymatrine.

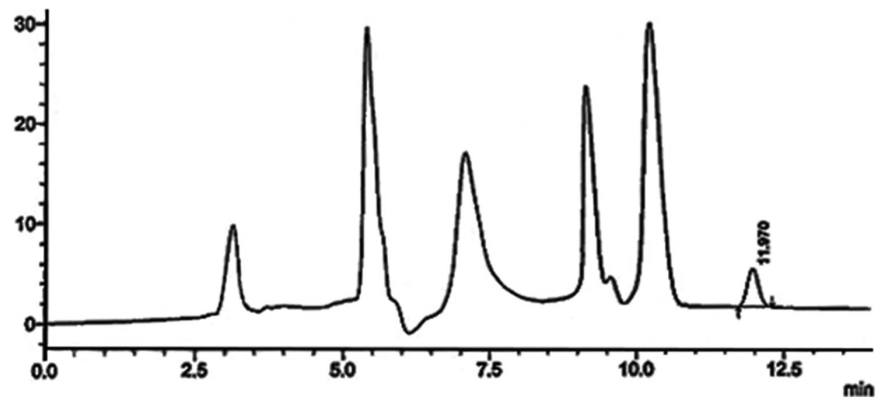


Fig. 10. Chromatograms for spiked water samples with 40 µg/mL oxymatrine.

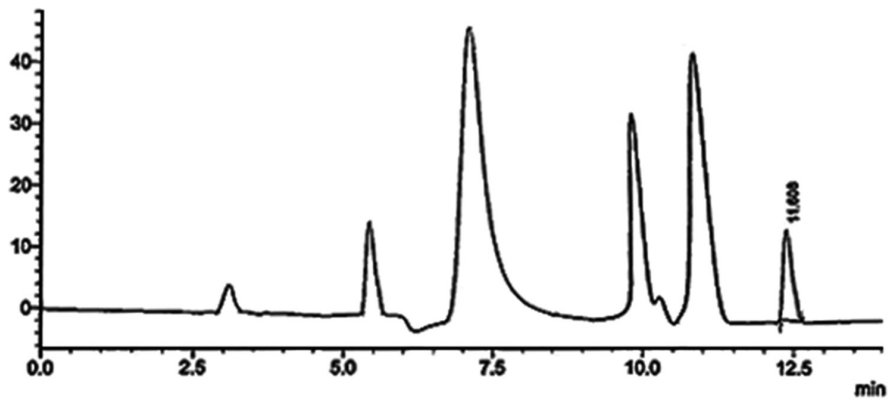


Fig. 11. Chromatograms for spiked water samples with 40.0 µg/mL oxymatrine.

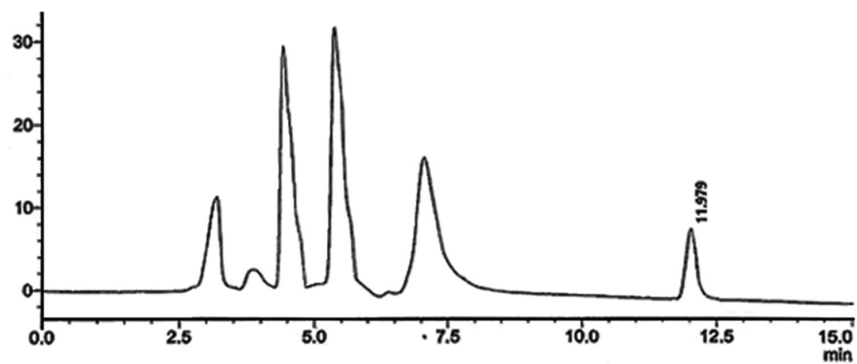


Fig. 12. Chromatograms for 30.0 µg/mL oxymatrine formulations.

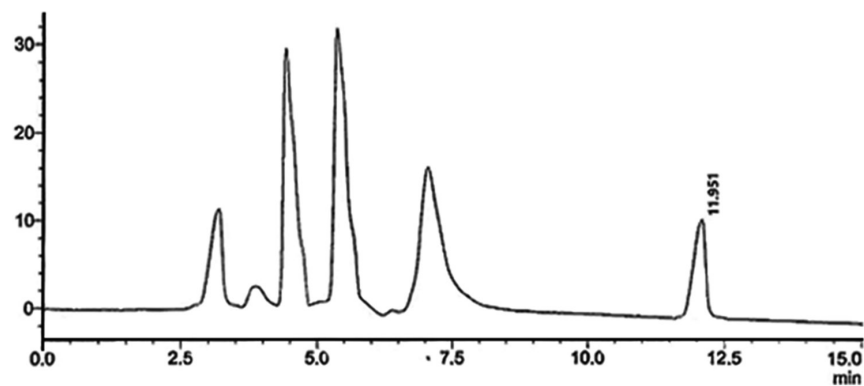


Fig. 13. Chromatograms for 60.0 µg/mL oxymatrine formulations.

determination of oxymatrine in spiked water samples and formulation.

X. CONCLUSION

Although this pesticide is widely and repeatedly used in the treatment of crops in the regions from which the samples collected, which may cause the accumulation of oxymatrine in water samples, leading to influence farmers in those areas, the applied method explained that there is no high level of oxymatrine residual in all water samples collected from those different agriculture areas in Kerbala city, Iraq. On the other hand, high accuracy was obtained for the determination of oxymatrine in spiked water samples and also in the formulation of oxymatrine that used in the field, as a result this method can be used successfully to follow up oxymatrine residual in the environmental samples.

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