WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 5, 135-143 Research Article ISSN 2278 – 4357

APPLICATION OF GC-MS IN DETERMINATION OF MALATHION IN ENVIRONMENTAL SAMPLES.

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Article Received on
06 March 2018,

Revised on 26 March 2018,
Accepted on 17 April 2018,

DOI: 10.20959/wjpps20185-11584

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ABSTRACT

A sensitive gas chromatography mass spectrometry (GC-MS) method was developed for determination of malathion in water samples. In this study, determination of malathion has been carried out according to standard method for water and wastewater analysis. Samples of collected water were drinking water, agriculture stream water, large stream water and shallow ground water from three governorates in Egypt: Assiut, Beni Suef and Sharkia. Malathion was extracted by liquid- liquid extraction and analyzed by GC-MS. The chromatographic separation was performed using a ZB5 column (30 m ×0.53 mm, 1.50 µm), and helium as a carrier gas. The limit of detection and limit of quantification for malathion were 0.62 and 2.07µg/L respectively. The intra- and inter-day precisions were lower than 2.28% while the accuracy ranged from 99.47% to 102.27%. Finally, the proposed method was successfully applied for determination of malathion in water samples. Malathion was observed in some agricultural streams and shallow groundwater agricultural areas.

KEYWORDS: Determination, Malathion, Water samples, GC-MS method.

INTODUCTION

Malathion(S-1,2-bis(ethoxycarbonyl)ethyl-O,O-dimethyl phosphorodithioate) is a widely used organophosphorus pesticide for the control of household and poultry pests, pests in

vegetables, field crops, fruits and domestic animals.[1] Malathion has a molecular formula of $C_{10}H_{19}O_6PS_2$ and molecular weight of 330.35 g/mol. Structural formula of malathion is shown in Fig. 1. Determination of malathion in environmental water samples usually requires the application of sample preparation procedures to extract the analyte from the aqueous solution and bring it to a suitable concentration level prior to final GC analysis.[2] Liquid–liquid extraction (LLE) is still the most common sample preparation approach. Malathion can be found in surface waters such as streams, and sometimes in well water.[3] Bacteria in the soil may break down malathion and sunlight can break it down in the air.[3] Hydrolysis is the main route of degradation in alkaline aerobic conditions.[4] Metabolites resulting from hydrolysis include malaoxon, malathion alpha and beta monoacid, diethyl fumarate, diethylthiomalate, o,o-dimethylphosphorodithioic acid, diethylthiomalate, and o,o-dimethylphosphorothionic acid.[4] Literature survey revealed a colorimetric method for the estimation of malathion,[5] Malathion degradation by soil isolated bacteria and detection of degradation products by GC-MS,[6] Kinetic investigation of malathion degradation in water,[7] SPE-GC–MS for determination of organophosphorus pesticides in underground Water,[8] (SPME / GC- MS) method for determination of malathion residues in wheat,[9] monitoring of pesticides water pollution in the Egyptian River Nile,[10] and determination of organophosphorus pesticides in surface water by SPE-GC–MS.[11] The aim of this study was to determine malathion in water samples by GC-MS method.

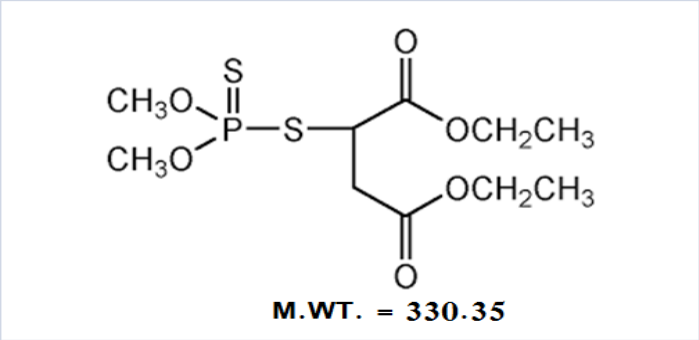

Figure 1: Structural formula of Malathion.

MATERIALS AND METHODS

Chemicals and Reagents

Malathion 95% was kindly provided by Egyptian international center for import. n-Hexane (99.0%), methylene chloride (99.9%), acetone (99.99%), and anhydrous sodium sulfate (99.99%) all these chemicals were of analytical grade and all were purchased from Sigma-Aldrich (Steinheim, Germany).

Instrumentation

Gas chromatography (GC)

The chromatographic analysis was performed by the GC Perkin Elmer Clarus 500 model equipped with a mass detector (Perkin Elmer Technologies, America), and a column compartment. The chromatographic separation was achieved on a Zebron ZB5 column (30m, 0.53 mm, 1.50 µm, Phenomenex, USA). The column was operated by a flow rate of 1 mL min^{-1} and an injection volume of 2-3 µL. The carrier gas was helium. The mass spectrometry ionization modes were electron ionization (standard) positive/negative chemical ionization (optional), compounds leaving the GC column are fragmented by electron impact. The charged fragments are detected, and the subsequent spectrum obtained can be used for identify the molecule. Fragmentation patterns are reproducible, and can be used to produce quantitative measurements. The control of the GC system and data processing were performed using PerkinElmer TurboMass™ GC/MS software.

Optimization of chromatographic condition

The oven temperature program was as follows: initial temperature maintained at 100 °C for 1 min, raised to 150 °C at a rate of 25 °C/min, hold 1 min, then raised to 260 °C at a rate of 5 °C/min, hold 3 min, and the injector temperature was maintained at 200 °C. Ethyl acetate was used as diluent. The Chromatograms obtained in these operating conditions for diluent, standard solution of malathion was shown in Fig. 2 and GC-MS spectrum of malathion was shown in Fig. 3.

Analytical method

Preparation of standard stock solution

Standard stock solution of malathion was prepared in ethyl acetate at a concentration of 9.5 µg mL^{-1}. Malathion working solutions in the desired concentration range was prepared by appropriate dilution of standard stock solution with ethyl acetate. The stock solution was prepared once a month, kept at 2–8 °C in a refrigerator and brought to room temperature before use.

Collection of samples

A total of 36 water samples were collected in amber glass bottles in August and September 2017. Samples of collected water were drinking water, agriculture stream water, large stream water and shallow ground water from three different areas Al-Badari in Assiut, Ahnasia in Beni Suef and Saoud in Sharkia (12 samples each).

Sampling

Water samples 2.5 L were collected in glass bottles at 50 cm below water level. Waters were collected by qualified personnel using standard sampling field protocol. The bottles were covered with screw caps and the samples were then stored at 4 $^{\circ}$C until extraction and analysis.

Liquid – Liquid Extraction of samples

A measured volume of 1 L of the sample was transferred in a 2 L separation funnel and was extracted twice with 60 mL 15% methylene chloride in hexane. The samples were shaken vigorously; the organic layer was taken and dried over anhydrous sodium sulfate and evaporated to dryness in a rotating evaporator. The residue was dissolved in 10 ml with ethyl acetate. 3µl of each extracted solution was injected into the gas chromatography.

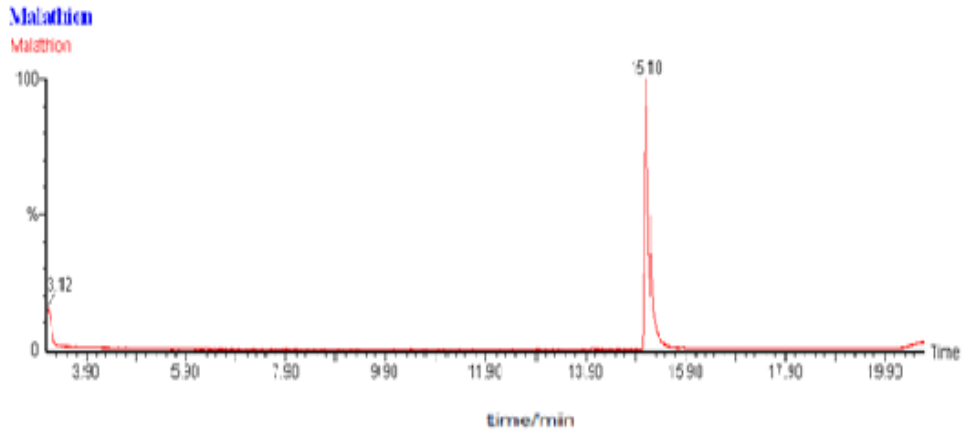

Figure 2: GC- MS chromatogram of malathion 95 ng mL^{-1}.

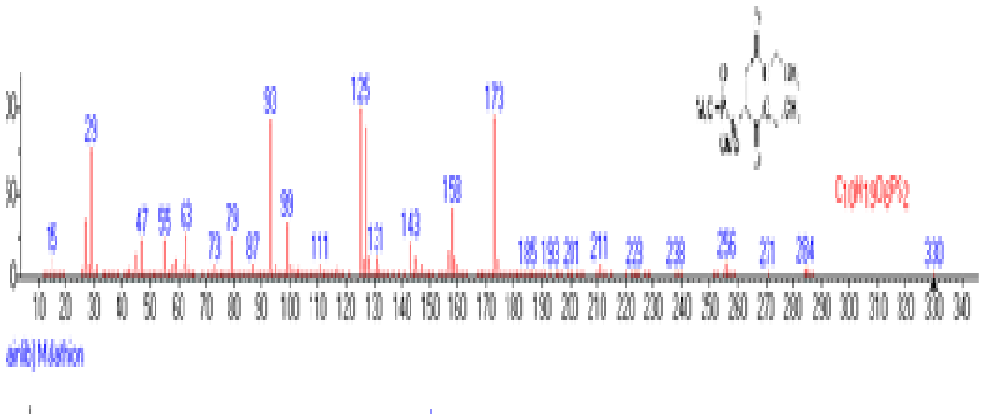

Figure 3: GC-MS spectrum of malathion.

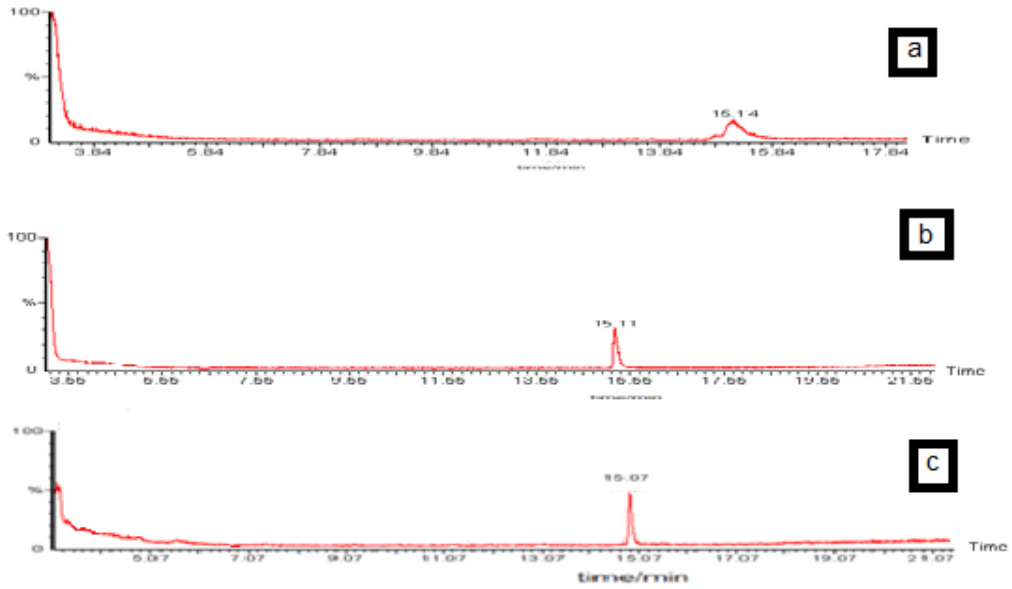

Figure 4: GC-MS chromatograms of Malathion in water samples: (a) Al badari agricultural stream sample in Assiut, (b) saoud agricultural stream sample in Sharkia and (c) saoud shallow groundwater sample in Sharkia.

Method validation

Linearity. Aliquots of 2 µL of analyte standard solution at six different concentrations (9.5–95 ng mL^{-1}) were injected into GC-MS system. The procedure was carried out in triplicate for each concentration. The analyte peak area obtained was plotted against the corresponding concentration of the analyte.

Accuracy and precision. Accuracy and precision were evaluated at three different concentration levels (9.5, 47.5 and 95 ng mL^{-1}) within the same day to obtain repeatability (intraday precision) and over three different days to obtain intermediate precision (inter-day precision). The accuracy and precision were calculated and expressed in terms of percent recovery and standard deviation, respectively. The Results were given in Table 1.

Limit of detection and limit of quantification. The limit of detection (LOD) is minimum amount of analyte in sample detectable and larger than uncertainty associated with it and the limit of quantification (LOQ) is amount quantitatively measured with suitable precision and accuracy. LOD and LOQ were determined by signal to noise ratio method.

Specificity. The specificity of the proposed method was assured by applying the GC-MS method in qualitative and quantitative determination of malathion in water samples according to standard method for water and waste water analysis.[12]

RESULTS AND DISCUSSION

Method development

Optimization of mass spectrometry parameters and chromatographic conditions was achieved to develop and validate a selective and rapid assay method for the determination of malathion in environmental samples.

Method validation

Linearity, LOD and LOQ. satisfactory linearity ($r \geq 0.999$) was obtained for malathion over the concentration range of 9.5–95 ng mL^{-1}. The analytical parameters of the proposed methods are summarized in **Table 2**. The limit of detection and the limit of quantification were calculated using the following equations.[13]

$$\mathbf{LOD} = c_s \frac{3}{S/N}$$

$$\mathbf{LOQ} = c_s \frac{10}{S/N}$$

Where S/N is the average signal to noise ratio and C_s is the concentration of the injected analyte. The estimated limits were verified by analyzing a suitable number of samples containing the analyte at the corresponding concentrations.

Accuracy and precision. Table 1 summarizes the intra- and inter-day accuracy and precision of Malathion at three different concentration levels (9.5, 47.5, and 95 ng mL^{-1}) on the same day (n = 3) and on consecutive days (n = 3).The RSD% values ranging from 0.52% to 1.91% for intra-day precision and from 0. 67% to 2.28% for inter-day precision studies, respectively, confirmed that the method was sufficiently precise. The intra- and inter-day recoveries ranged from 99.47% to 102.27%; these results demonstrated that both intra- and interday precision values were all within the acceptance variability limits. The proposed method has been successfully applied for the determination of malathion in water samples and the results are tabulated in Table 3.

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The specificity was evaluated from the GC-

MS chromatogram. The GC-MS chromatogram of the samples shows that the method has sufficient specificity to resolve all related substances and the malathion from each other as shown in Fig. 4. Furthermore, the mass detector also showed excellent mass purity for malathion. Table 3 contains a summary of malathion concentrations in samples collected during the study.

Robustness

Robustness is an important aspect of method validation for chromatographic methods. The influence of small deliberate changes in the operations (variations) of the analytical procedure is evaluated from measured or calculated responses. This is to verify that the method performance is not affected by typical changes in normal experiments. Robustness was evaluated by changing the flow rate (1 ± 0.1 mL/min). The effect of GC oven temperature was studied at 100 ± 1 °C (optimized temperature was 100 °C). The measured response variances were the % RSDs. The % RSDs of peak area for malathion were given in Table 4. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust.

Table 1: Intra –day and Inter –day accuracy and precision of malathion.

Drug	Concentration (ng/ml)	The concentration found (ng/ml) ± SD; RSD			
		Intraday precision	accuracy	Interday precision	accuracy
	9.5	9.56±0.18; 1.88	100. 60	9.45±0.16; 1.69	99.47
Malathion	47. 5	47.74±0.91; 1.91	100.49	47.41±1.08; 2.28	102.27
	95	95.12±0.49 ; 0.52	100.13	94.83±0.64; 0.67	99.82

Table 2: Analytical parameters and linear regression data of malathion.

Parameter	
Linearity range (ng mL-1)	9.5-95
LOD (ng mL-1)	0.62
LOQ (ng mL-1)	2.07
Regression equation*	
Correlation coefficient	1
Slope (b)	84897
Intercept (a)	-16575

*$Y = a + bC$, where Y is the peak area and C is the concentration in ng mL^{-1}.

Table 3: Mean ± SD of malathion residue levels (µg/L) in water samples.

Sites sampled	Found concentration (mean ± SD)			
	Drinking water	Agricultural stream sites	Large stream and River Nile	Shallow groundwater agricultural areas
Assiut	Not detected	14.77±0.30	Not detected	Not detected
Sharkia	Not detected	27.51±0.46	Not detected	21.22±0.17
Beni Suef	Not detected	Not detected	Not detected	Not detected

Table 4: Robustness data.

Parameter	Validation	%RSD of the area Malathion
Flow rate	0.9 ml/min (low) 1.1 ml/min (high)	0.91 1.69
GC oven temperature	99 ºC (low) 101 ºC (high)	1.59 0.69

CONCLUSION

A validated, sensitive and accurate GC-MS analytical method was developed for the analysis of malathion in water samples. The method was fully validated according to the ICH guidelines and presented good linearity, accuracy, precision and specificity. The LOD and LOQ Values were established by using signal to noise ratio method. Malathion was observed in agricultural streams samples of Al badari in Assiut and saoud in Sharkia and shallow groundwater samples of soaud in Sharkia due to the use of malathion as a pesticide near these areas. However, it was not observed in drinking water and large streams water in the three areas. The proposed method can be successfully applied for determination of malathion in water samples using GC-MS method.

ACKNOWLEDGMENT

the author acknowledges to staff members Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy Al-Azhar University, Cairo, Egypt for their co-operation throughout the whole work. also acknowledgment goes to Research Laboratories, Ministry of health, Giza, Egypt for providing the necessary facilities and support in completing this work.

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