

Rapid and Inexpensive Densitometric Method, Using TLC Plates, for Quantification and Determination of Nine Pesticides in Water

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ABSTRACT

Nine pesticides of commonly used in Gaza Strip were tested in this work for optimal separation onto silica gel TLC plates in acetone/ cyclohexane, 1:2 (v/v), as a mobile phase to develop a new densitometric method for quantification and determination of these pesticides in water samples. These pesticides are: abamectine, atrazine, benomyl, cymoxanil, diuron, imidacloprid, linuron, penconazole, and propazine. Calibration plots were linear between 25 to 1000 ng/spot for all pesticides with a correlation coefficient, *r*, between 0.9920 and 0.9993. Recoveries after enrichment of real water samples on Bakerbond RP-C18 cartridges were between 79.4 and 103.7 % at a relative standard deviation between ± 1.92 and 3.68 %. Detection limits LOD of 25 - 100 ng per spot for eight of the pesticides were reached, which meet the European limits for pesticides in drinking water (100 ngL⁻¹), while penconazole has an LOD value of 200 ngL⁻¹ because of its low UV-absorption coefficient.

Key words: pesticides, TLC, solid phase extraction, Gaza Strip.

1. INTRODUCTION

In modern agricultural practices pesticides are used mainly for the protection of crops against pests and diseases. However, they have several disadvantages as a result of their undecimate application: they are hazardous to human health and animals, they do not completely solve pest problems, they often contaminate food, water and air.

Pesticides are considered priority pollutants in Gaza and, with the expanding use of greenhouses; Palestinian agriculture is becoming increasingly dependent on chemical pesticides and fertilizers. Leaching of pesticides, a common and growing problem in major agricultural regions including Gaza Strip, Palestine¹, from soil can lead to the contamination of the Gaza groundwater².

Issa concluded in his survey that the misuse of pesticides by the general public increased the level of soil and water contamination across Gaza³. According to Safi⁴, Gaza Strip consumed more than 393.3 tons of pesticides and more than 900 tons of methyl bromide are used annually to protect the major crops, including vegetables, citrus, olives and grapes in 1999⁵⁻⁶. More than 100 metric tons of formulated pesticides (about 75 pesticides) are used annually in Gaza Strip. It was found that 19 of these pesticides, that have been used, are internationally suspended, cancelled and banned pesticides⁷⁻⁸.

Shomar *et al*² showed that, more than 92% of common pesticides in groundwater were much lower than their allowable limit of the World Health Organization (WHO). However, the municipal groundwater wells showed better quality as they are located in the residential areas than the private wells in the agricultural regions. Atrazine, atrazine-desisopropyl, propazine, simazine, DDT, DDE, DDD and triadimenol were detected in some wells in Gaza Strip. Aaron *et al*⁹ found that the relatively high pesticide contamination levels in ground waters and surface waters: 0.1 - 0.3 $\mu\text{g L}^{-1}$ in US ground waters, 0.03 - 0.5 $\mu\text{g L}^{-1}$ in European ground waters, while according to the above study², approximately 1.2 - 3.5 $\mu\text{g L}^{-1}$ for some pesticides were detected in ground water of Gaza Strip. The widespread use of pesticides in the Gaza Strip and their presence in varying and troubling quantities makes us worry about the future of groundwater in the Gaza Strip and the risk of the impact of these pesticides on the soil, plants, humans and the environment. For example, methyl bromide, which is used extensively in Gaza, causes fetus deformations, eye infections and dermatitis⁴. Organochlorine pesticides used in Gaza cause breast cancer¹⁰. Another study conducted by Safi⁴ showed that heavy misuse of pesticides in the Gaza environment correlated with the growing incidence of cancer. It is ironic that pesticides are banned or restricted in many countries are being marketed and used in Gaza because of lack proper assessment and monitoring programs¹¹. Also lack of awareness among pesticides' shop owners, farmers and public increased the level of soil and water contamination across Gaza³⁻¹².

This work contributes to development of a new densitometric method, using TLC plates, appropriate for monitoring of nine pesticides, mostly used in the Gaza Strip in Palestine, in water samples. The proposed method is rapid, simple and inexpensive. These pesticides are: abamectine, atrazine, benomyl, cymoxanil, diuron, imidacloprid, linuron, penconazole, and propazine. Solid phase extraction (SPE) conditions was optimized to obtain good recovery for investigated pesticides. Also, the LOD for them was measured.

Although there is a wide choice of other chromatographic methods for pesticides analysis (GC, HPLC), thin layer chromatography TLC remains a valid and simple analytical procedure for qualitative detection and quantitative determination of pesticides and their metabolites in the environmental samples¹³. TLC is being used more in the recent past, and is the object of an official method¹⁴. Among several published, this method has proven every efficient and

reliable in the screening of the various classes of pesticides.

From many literature reviews it was found that the nine investigated pesticides had not been completely separated together on one stationary phase of TLC plates. Only some of the investigated pesticides were separated on several stationary phases and a number of visualization techniques were tested in order to enhance the UV – absorption coefficient¹⁵⁻³⁷.

2. EXPERIMENTAL

The investigated pesticides were obtained from Dr Ehrenstofer GmbH, Augsburg, Germany; the compound and purities were: abamectine, atrazine, benomyl, cymoxanil, diuron, imidacloprid, linuron, penconazole, and propazine. RP-C18 Bakerbond spe, 3 mL, 500 mg cartridges from J.T.Baker (Gross-Gerau, Germany) were used to enrich water samples. All solvents for SPE and mobile phase components were of analytical grade and were obtained from Merck, Darmstadt, Germany.

Standard solutions of investigated pesticides were prepared by dissolving 2 mg of each in 1 mL in suitable solvents to achieve best solubility as follows: abamectine, cymoxanil, diuron, linuron and atrazine were dissolved in ethyl acetate, benomyl in ethanol, penconazole in dichloromethane, propazine in toluene, and imidacloprid in dichloromethane. The prepared standard solutions were then diluted stepwise in the range between 5 and 2000 ngL⁻¹. The solutions were stored under refrigeration at +4°C for several days.

2.1 Solid-Phase Extraction

One liter of drinking water was adjusted to a pH 5-7 using an Orion model 330 pH-meter and spiked with a solution of the nine investigated pesticides (abamectine, atrazine, benomyl, cymoxanil, diuron, imidacloprid, linuron, penconazole, and propazine). The water sample was enriched on C18 Bakerbond spe, 3 mL, 500 mg cartridges as follows: The cartridges were firstly washed with 10 mL methanol and then conditioned with 5 mL water. The column should not be allowed to run dry between and after the conditioning steps. After allowing the sample to pass through the cartridges with a rate of 2-3 mL / min the cartridges were washed with 3 mL water and let drying for 20 min under vacuum. For the elution of investigated pesticides different volumes of methanol and acetonitrile were tested separately in 1 mL portions. Using this procedure it was found that 5 mL of the acetonitrile was sufficient enough to elute the most pesticides from the solid material of the cartridges.

Each cartridge was finally eluted with 5 mL acetonitrile, evaporated to dryness by a stream of nitrogen gas and re-dissolved in 100 µL acetonitrile. The solution obtained was used for TLC analysis. The same procedure was applied for blank sample.

The above SPE developed method was capable to remove all interferences in drinking water such as the high concentration of nitrates and chlorides and other dissolved natural organic materials. The chromatogram in **Fig-1B** of a blank sample was obtained after SPE enrichment showed no interfering peaks.

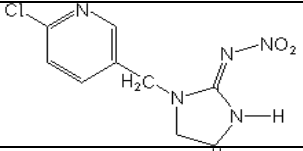
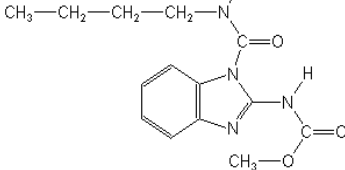
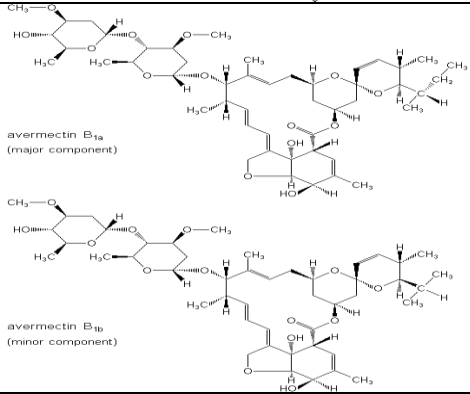
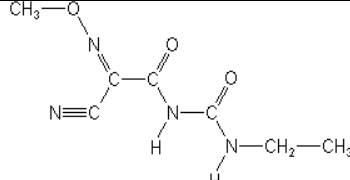
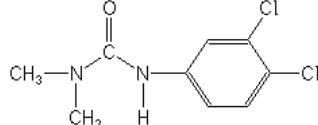
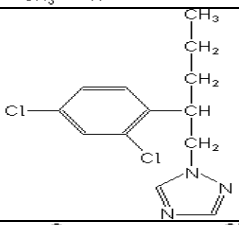
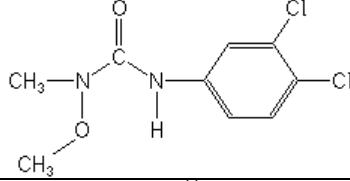
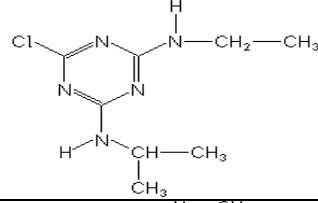
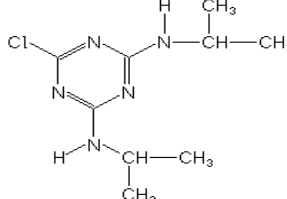
2.2 Chromatography

Chromatography was performed on 20 cm x 20 cm plates pre-coated with 0.2 mm layer of silica gel 60 F₂₅₄ (Macherey-Nagel, Germany). For quantitative analysis standard solutions (2 µL) and sample solutions (2 µL) were applied to the plates as spots by using disposable micropipets; otherwise the entire SPE eluate (100 µL) was applied as a band, by use of the Linomat IV (Camag), with distance of 1 cm between the bands (0.5 cm wide) and a distance of 2 cm from the bottom and both edges of the TLC plate. The nine investigated pesticides were separated by ascending one-dimensional development, in a saturated chamber, with acetone/ cyclohexane, 1:2 (v/v), as a mobile phase. The development distance was 10 cm and the run time 15 min. The spots or bands were located by viewing under a universal UV lamp (Vilber Lourmat, France) at $\lambda = 254$ nm. Pesticides were determined with a Shimadzu CS-9301 dual Wavelength, flying-spot scanning densitometer in reflectance mode using deuterium lamp at $\lambda = 270$ nm. The beam size was 0.4 mm x 16 mm, the deuterium lamp was used for zero set mode at start, 15-point smoothing, and the calibration via peak area. The peak find filter was 50 and resolution during data collection was 0.04.

3. RESULTS AND DISCUSSION

Different combinations of solvents on silica gel TLC plates were tested for optimum separation of the nine investigated pesticides (abamectine, atrazine, benomyl, cymoxanil, diuron, imidacloprid, linuron, penconazole, and propazine) (**Table-1**). Acetone-cyclohexane, 1:2 (v/v), as a mobile phase in a saturated chamber proved to be optimum for separation of the nine investigated pesticides. The UV absorption wave lengths of the nine pesticides varied from 222 to 279 nm. However, the best chromatogram of the scanned separated pesticides on the TLC plate was obtained at a wave length of 270 nm. **Figure 2** shows the hR_F values of all investigated pesticides. These analyses were conducted in triplicates and the results showed at the mean values. The relative standard deviations for the three determinations were in the range of 1.8 – 3.2%. **Figure 1** illustrates the separation of the nine pesticides on silica gel TLC plate (development distance 10 cm) after SPE of spiked real water sample (obtained from university well). It was observed that shorter development distance of the TLC plate lead to overlapping of the pesticides while development to longer distance gave bad resolution because of the high diffusion efficiency of acetone.

Table-1: The structures and the chemical names of the nine investigated pesticides.

Pesticide	Structural formula	Chemical name
Imidacloprid		(2 <i>E</i>)-1-[(6-chloro-3-pyridinyl)methyl]- <i>N</i> -nitro-2-imidazolidinimine
Benomyl		methyl[1-[(butylamino)carbonyl]-1 <i>H</i> -benzimidazol-2-yl]carbamate
Abamectin		avermectin B ₁
Cymoxanil		2-cyano- <i>N</i> -[(ethylamino)carbonyl]-2-(methoxyimino)acetamide
Diuron		<i>N'</i> -(3,4-dichlorophenyl)- <i>N,N</i> -dimethylurea
Penconazole		1-[2-(2,4-dichlorophenyl)pentyl]-1 <i>H</i> -1,2,4-triazole
Linuron		<i>N'</i> -(3,4-dichlorophenyl)- <i>N</i> -methoxy- <i>N</i> -methylurea
Atrazine		6-chloro- <i>N</i> -ethyl- <i>N'</i> -(1-methylethyl)-1,3,5-triazine-2,4-diamine
Propazine		6-chloro- <i>N,N'</i> -bis(1-methylethyl)-1,3,5-triazine-2,4-diamine

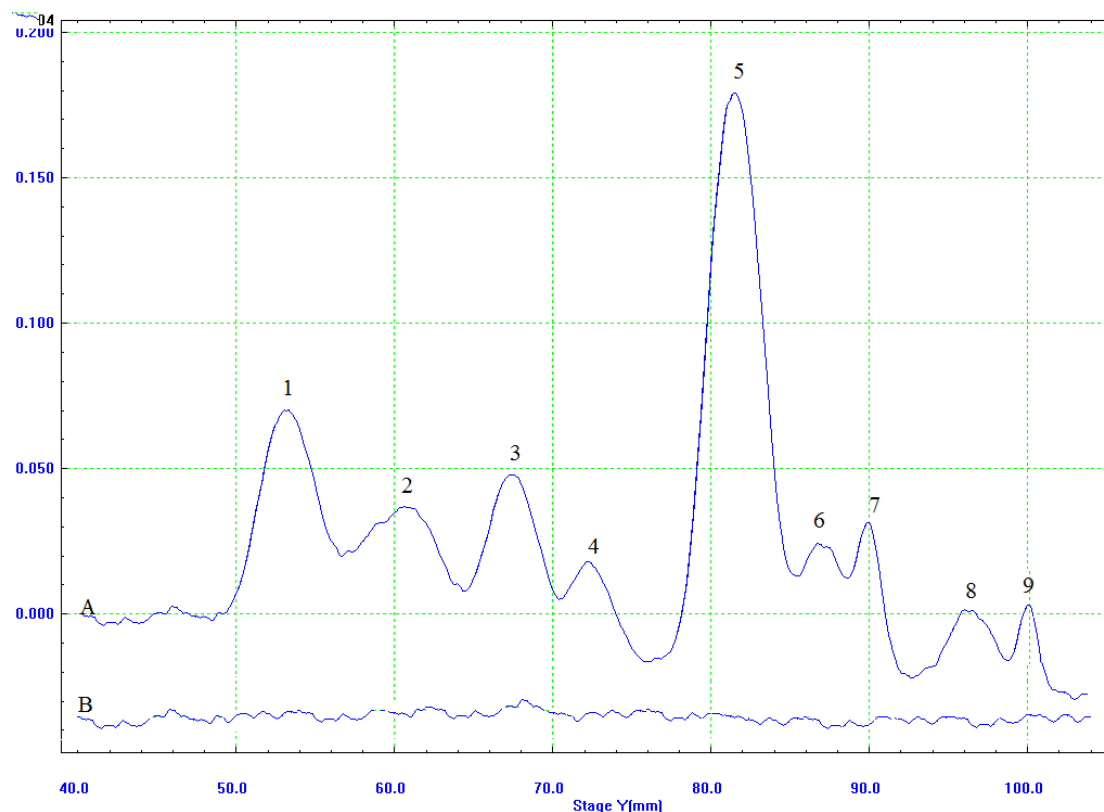


Fig-1:A: Densitogram obtained from separation of the nine investigated pesticides on silica gel TLC plate after enrichment of spiked real water sample; 1: Propazine, 2: Atrazine, 3: Linuron, 4: Penconazole, 5: Diuron, 6: Cymoxanil, 7: Abamectine, 8: Benomyl, 9: Imidaclopride; mobile phase: acetone / cyclohexane 1: 2 (v/v); developing distance 10 cm. B: Densitogram obtained from blank extract of real water sample (from university well).

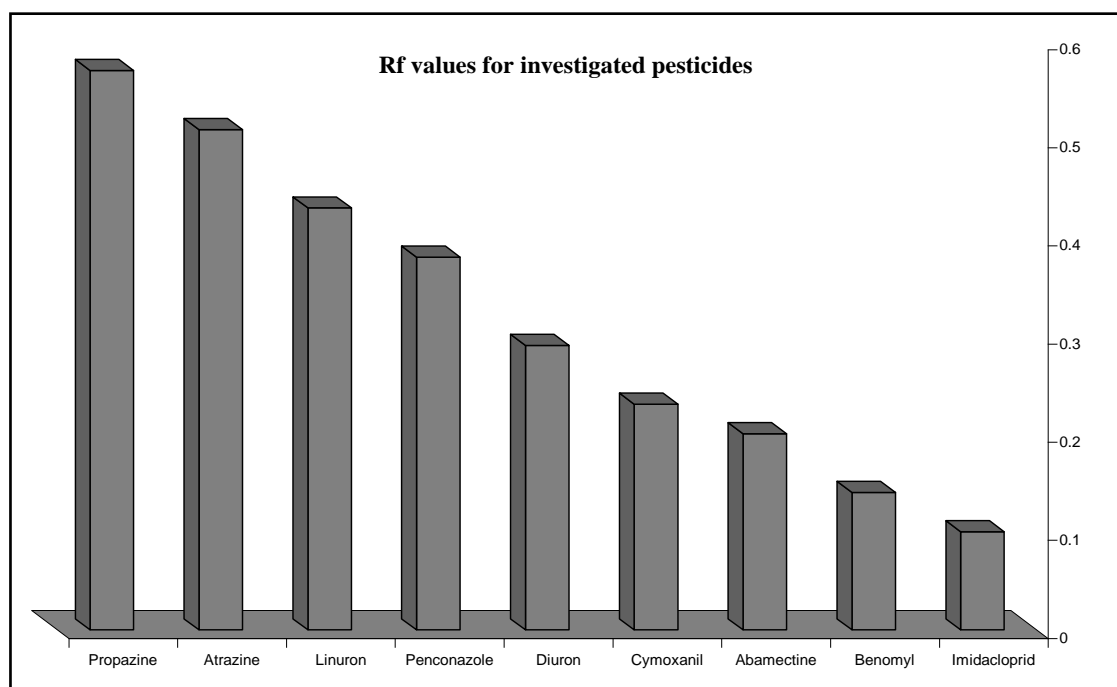


Fig-2: The hR_F values of all investigated pesticides.

Calibration plots were constructed for each pesticide under investigation by plotting peak area against the concentration of pesticide applied to the TLC plate (not shown). The spots or bands were scanned with the help of the CS-9301 Shimadzu densitometer in the cross lateral direction of the developing axis under the same chromatographic conditions mentioned above. The plots were linear in the range between 25 and 1000 ng, with correlation coefficient, r , between 0.9920 and 0.9993. A signal-to-noise ratio of 3 to 1 was chosen to define the detection limit for each investigated pesticide.

Table-2: The correlation coefficient, regression equations, r, mean Rf – values, LOD values and the specific UV absorption wavelength for nine investigated pesticides

Pesticide	Wavelength λ (nm)	correlation coefficient (R^2)	regression equations	LOD (ng L^{-1})
Imidacloprid	270	0.9989	$y = 0.1518x + 8.0577$	100
Benomyl	279	0.9992	$y = 0.2521x + 15.946$	25
Abamectine	244	0.9991	$y = 0.0479x + 6.1537$	100
Cymoxanil	244	0.9920	$y = 0.076x + 13.32$	100
Diuron	250	0.9989	$y = 0.2479x + 5.2442$	25
Penconazole	220	0.9987	$y = 0.1261x - 12.504$	200
Linuron	250	0.9992	$y = 0.1799x + 0.6208$	25
Atrazine	222	0.9993	$y = 0.3556x + 4.0515$	50

These results are summarized in **Table 2**. A detection limit of 25 - 100 ng per spot (or band) for eight of pesticides were reached, which meet the European limits for pesticides in drinking water (100 ng L^{-1}). However, the penconazole pesticide has weaker absorption to UV light, which lead to higher LOD value (200 ng L^{-1}). In this case a 2 L sample for SPE could solve the problem.

Table-3: Recoveries, standard deviation (SD) and relative standard deviation (RSD) after enrichment of a liter water life sample spiked with the investigated pesticides (100 ng) on RP-C18 bakerbond cartridges.

Pesticide	Areas	Average	SD	RSD %	Recoveries %
Imidacloprid	523.5	520.03	10.5	2.01	94.8
	508.2				
	528.4				
Benomyl	285.1	284.7	5.9	2.07	85.9
	278.6				
	290.4				
Abamectine	218.6	223.4	6.25	2.79	79.8
	230.5				
	221.1				
Cymoxanil	267.8	270.5	9.05	3.34	92.5
	280.6				
	263.1				
Diuron	183.9	185.2	5.8	3.1	95.8
	180.2				
	191.6				
Penconazole	188.9	188.7	6.95	3.68	79.4
	181.7				
	195.6				
Linuron	154.3	155.2	2.99	1.92	103.7
	158.5				
	152.7				
Atrazine	476.4	480.8	16.3	3.4	95.3
	467.2				
	498.9				
Propazine	338.9	335.2	7.5	2.2	80.5
	326.6				
	340.2				

To test the applicability of this method to the determination of the studied pesticides in drinking water, a sample of pesticide-free drinking water (1 L), obtained from university well, was spiked with the nine pesticides in study (0.1 μg of each), adjusted to pH 5-7 and the sample was enriched by C18 Bakerbond spe, 3 mL, 500 mg cartridges. After drying, the cartridges were eluted as described above. The eluate was evaporated to dryness and re-dissolved in 100 μL acetonitrile. The complete volume could be applied to the TLC plate by means of Linomat IV with standard solutions, then scanned by densitometer and determined quantitatively. The results obtained from the SPE material are listed in **Table-3**.

4. CONCLUSION

In this work, TLC separation of nine investigated pesticides, commonly used in Gaza Strip, on silica gel plate, and their densitometric determination, are described. Recoveries after enrichment of real water samples by solid phase extraction were optimum for quantitative determination.

The method developed is rapid, inexpensive, reliable and sensitive enough to meet international limits ($0.1 \mu\text{g L}^{-1}$).

The nine investigated pesticides were separated on silica gel TLC plate in acetone/cyclohexane 1:2 (v/v) as a mobile phase at a developing distance of 10 cm. A linearity range between 25 to 1000 ng/spot was achieved with a correlation coefficient between 0.9920 and 0.9993. The recoveries obtained were between 79.4 and 103.7 % at a relative standard deviations between 1.92 and 3.68 %.

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