CHROMATOGRAPHIC CONDITIONS FOR DETECTION OF THE NEONICOTINOID INSECTICIDES THIAMETHOXAM AND CLOTHIANIDIN, AND THE EFFECT OF SOME ENVIRONMENTAL FACTORS ON THEIR STABILITY

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INTRODUCTION

Neonicotinoid insecticides represent the fastest growing class of insecticides introduced to markets since the launch of pyrethroids (Nauen and Bretschneider, 2002). The current market share of the chemicals of this class is above 600 million Euro per year, including imidacloprid as the biggest selling insecticide world wide. Other neonicotinoids commercialized since the introduction of imidacloprid are acetamiprid, nitenpyran, thiamethoxam, thiacloprid, clothianidin and dinotefuran (Takahashi *et al.,* 1992; Maienfish *et al.,* 1999; Elbert *et al.,* 2000; Nauen *et al.,* 2001; Ohkawara *et al.,* 2002; Kodaka *et al.,* 2002 and Tomizawa and Casida, 2003).

Neonicotinoids are active against numerous sucking and biting pest insects, including aphids, whiteflies, thrips, leaf miners, beetles and some lepidopteran species (Elbert *et al.,*1998). Their physicochemical properties render them useful for a wide range of application techniques.

They show good activity against insects resistant to other classes of insecticides (Denolm *et al.,* 2002). Thiamethoxam was the first commercially available second- generation neonicotinoid which belongs to the thianicotinyl sub-class. It has broad-spectrum activity against a variety of important pests in many crops. It is used rates, in low flexiblity in application methods (foliar, soil and seed treatments), efficacy, safety profile which make it well-suited for IPM programmes (Maienfisch *et al.,* 2001).

Efficient method development and some times successful troubleshooting of certain problems in HPLC mainly depend on the understanding of the retention mechanism and the way how different parameters influence the separation. Kazakevich (1996) discussed the retention dependencies in RP-HPLC using the surface interaction approach. They have to emphasize that all types of component behaviour in the column are related to the type and strength of its interaction with the adsorbent surface and of the interaction of the eluent molecules with the surface in addition to adsorbent surface chemistry.

Nauen *et al.*, 2003 reported that thiamethoxam was rapidly metabolized when applied to 5th instar *Spodoptera frugiperda* larvae and to cotton plants to clothianidin, which was the predominant neonicotinoid after application. In conclusion thiamethoxam is likely to be a precursor for clothianidin.

The aim of this work was to: 1- choose the suitable chromatographic operating condition of TLC and HPLC for detection and determination of thiamethoxam and clothianidin. 2- study the effect of temperature, uv-rays and sunlight on the persistence of thiamethoxam and clothianidin.

MATERIAL AND METHODS

The neonicotinoid insecticides tested in this study are thiamethoxam [3-(2-chloro-1,3-thiazol-5-ylmethyl) -5-methyl-1,3,5-oxadiazinan-4-ylidene (nitro) amine] and clothianidin [(E) – 1 - (2 -chloro-1, 3 thiazol – 5 - ylmethyl) – 3 – methyl – 2 - nitroguanidine].

Thin Layer Chromatography (TLC):

Glass chromatoplates 20×20 were covered with a thin layer 0.5 mm thick of silica gel GF 254, were used. The tested insecticides were spotted, then the plates were developed in glass jars using different-solvent systems (acetone, benzene, ethanol, ethyl acetate, hexane, methanol and methylene chloride. Also, solvent mixtures in a ratio 1:1 between acetone and another solvent (benzene, dichloromethane, ethyl acetate, hexane) were used. The developed spots were visualized under ultra violet lamp (UV) at wavelength 254 nm.

High Performance Liquid Chromatography (HPLC):

The chromatographic system consisted of Hewlett Packard (Hp series 1100), Quaternary pump model (G1311A), UV variable wavelength. Model (G1314A) monitored at the two wave length 255 and 265 nm for each of thiamethoxam and clothianidin. An ODS-Hypersil 5μm (20cm mm i.d) was used with 40°C column temperature. Thiamethoxam and clothianidin were eluted with four different solvent systems: methanol: water 20:80, methanol: water 30:70, methanol: 50 mM ammonium acetate 25:75 and acetonitrile: 0.1% acetic acid 25:75. A 20 µl injector was used at three different flow rates 0.5, 0.8, 1.0 ml/min. Under these conditions, the retention time (R_t) for thiamethoxam and clothianidin was tabulated.

Effect of ultra violet rays, sunlight and temperature:

Thiamethoxam and clothianidin were exposed to 254 nm UV lamp in the form of a dry film, in uncovered petri-dishes at a distance of 10 cm and a diameter of 5 cm. They were tested in triplicates for each exposure period, which were 2, 6, 12, 18, 24, 30 and 36 hours for thiamethoxam and 12, 24, 48, 72 and 96 hours for clothianidin.

80 µg thiamethoxam or clothianidin were exposed in uncovered petri dishes to direct sunlight (through daytime). Samples were removed away from direct sunlight after 1, 2, 4, 6, 8, 10, 12, 14, 16 and 18 hours for thiamethoxam and after 2, 3, 6, 12, 14, 18, 24 and 30 hours for clothianidin. Temperature degrees ranged from 32 to36 °C.

Both pesticides were subjected in the form of a dry film to 40, 50, 75 °C, in a dark electric oven provided with a thermometer, in uncovered petri-dishes (5 cm in diameter) containing 80µg thiamethoxam or clothianidin. The exposure periods were daily up to 7 days.

The half life time t½ for thiamethoxam and clothianidin was calculated according to (Moye *et al.*(1987).

$$t½ = \frac{In_2}{K} = \frac{0.6932}{K} \qquad K = \frac{1}{tx} \cdot In\,\frac{a}{bx}$$

Where: **K** = rate of decomposition **a** = initial residue **tx** = time
bx =residue at x time

RESULTS AND DISCUSSION

Detection of thiamethoxam and clothianidin by TLC:

The results in table (1) summarize the effect of different solvent systems on the R_f values of both insecticides. Non polar solvents including benzene, hexane and dichloromethane resulted in R_f values = zero for both insecticides. The developing of thiamethoxam and clothianidin differed in the different polar solvents. The highest R_f values obtained were 0.81 and 0.91 for thiamethoxam and clothianidin with acetone. The descending order of R_f values with these solvents were as follows: acetone, methanol, ethanol and ethyl acetate. In general the R_f values in polar solvents were higher for clothianidin than those of thiamethoxam.

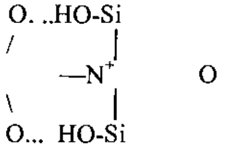
The discussion of the mentioned data will be based on the relation between the chemical structure of thiamethoxam and clothianidin and both the adsorbed and mobile phase.

TABLE (I)

R_f values of thiamethoxam and clothianidin using different solvent systems

Solvent System	R_f values	
	Thiamethoxam	Clothianidin
Benzene	0.0	0.0
Hexane	0.0	0.0
Dichloromethane	0.0	0.0
Acetone	0.81	0.91
Methanol	0.77	0.87
Ethanol	0.56	0.86
Ethyl acetate	0.40	0.62
Acetone:hexane 1:1	0.40	0.63
Acetone:dichloromethane 1:1	0.77	0.86
Acetone:benzene 1:1	0.64	0.74
Acetone:ethyl acetate 1:1	0.78	0.90

The chemical structure of the silica gel used in this work as adsorbent phase was reported to have free hydroxyl groups on the surface of its structure (Kicelev 1956). Klessenco (1969), studying the chromatographic procedure of nitro and dinitro aromatic pesticides stated that there is the hydrogen bond between oxygen of the nitro group and the proton of hydrogen atom (H^+) of the hydroxyl group on the surface of the adsorbent. In the case of the tested two insecticides thiamethoxam and clothianidin might have specificity due to the binding between the oxygen atoms of $-NO_2$ group and the proton of hydrogen atom (H^+) of the hydroxyl group on the surface of silica, showed that there is a hydrogen bond of the following type:



It may be another hydrogen bond between Cl atom of the both pesticides and H^+ of –OH group on the surface of silica Cl...HO-Si. The strength of these hydrogen bonds is large and therefore the R_f values of these two compounds were 0.0 in non-polar solvent such as hexane, benzene and dichloromethane (Table I). R_f values of thiamethoxam were lower than those of clothianidin in tested polar solvents. This may be due to the presence of group having positive inductive effect (I^+) such as $-CH_3$ in thiamethoxam molecule and also, the presence of heterocyclic ring containing O, C, N, N atoms in the same compound leads to withdrawing electrons from either $-CH_3$ or this ring which may increase the energy of hydrogen bond between the oxygen atom in the compound and H^+ of hydroxy group on the surface of silica gel. Consequently, the increase in conjugation between thiamethoxam and silica gel surface led to a decrease in the R_f values of thiamethoxam molecule than that of clothianidin.

Detection and Determination of thiamethoxam and clothianidin under different operating conditions of HPLC:

Table (2) contains the results of determining thiamethoxam and clothianidin pesticides under two different wave lengths (255 and 265 nm), with three flow rates for each of the four mobile phases tested.

Effect of flow rate:

In general the increase in the flow rates at all of the used mobile phases caused an obvious decrease in the resulting retention time, peak area and width of the peak. The effect of flow rate on the R_t using acetonitrile / acetic acid 0.1% at 255 nm appeared as follows on thiamethoxam: 8.06, 4.85 and 3.85 min. at flow rates 0.5, 0.8 and 1.0 ml/min., respectively. The corresponding R_t values for clothianidin were 9.49, 5.73 and 4.69 min. The rate of increasing the R_t according to the decrease of flow rates from 1.0 to 0.8 or 0.5 ml/min. were 1.28 and 2.05 times for thiamethoxam and were to great extent the same (1.22 and 2.02 times) for clothianidin.

Also, it was obvious that the area and width of peak decreased with increasing the flow rate. As in the case of acetonitrile/acetic acid 0.1% under wave length 255 nm, the peak areas were 2398.3, 1461.8 and 998.8 mAU for thiamethoxam and 1035.3, 784.8 and 551.9 for clothianidin. While the peak widths were 0.42, 0.26 and 0.18 for thiamethoxam and 0.43, 0.28 and 0.23 min. for clothianidin at the same flow rates, respectively. The rate of change in area varied with the change of flow rates. For thiamethoxam the rates were 1.64 and 2.40 and for clothianidin were 1.31 and 1.87 using acetonitrile/acetic acid 0.1%. But using water/methanol (70/30) gave a rate of 1.27 and 1.7 with thiamethoxam and 1.64 and 1.68 with clothianidin (Table 2).

The effect of increasing the flow rate on the detection limit (D.L.) varied. In some cases the D.L. was stable as in the case of thiamethoxam and clothianidin detection with acetonitrile /acetic acid 0.1% which was 0.5 ng in all of the tested flow rates. In other cases the D.L. values decreased with increasing the flow rate e.g. using methanol / water (30/70) with thiamethoxam under 265 nm gave 1.0, 1.0 and 0.5 ng with flow rates 0.5,0.8 and 1.0ml/min, respectively. The correspondingly values under 255 nm were 1.0, 0.5 and 0.5 ng, respectively. The same trend was obtained with clothianidin under both wavelengths.

These results were in agreement with Kazakevich (1996) who stated that the specific retention behaviour of solutes in adsorption chromatography at given eluent composition is governed mainly by the chemical nature of the silica surface. The synthesis and stability of surface bonded layers are also dependent on silica surface chemistry, types of the surface silanols, Si-OH and their reactivity. Most of the chromatographic properties of the silica surface are related to the interactions with silanol groups. It is important to note, that in HPLC the surface interactions are always competitive. Adsorbent surface is in the permanent contact with eluent. Eluent molecules interact with the surface adsorption sites according to their structure, polarity and ionisability. Analyte molecules will adsorb onto the silica surface (and so retained) only if their interaction is stronger than that of eluent molecules. Reversed-phase chromatography mainly utilizes non-specific hydrophobic (dispersive) interactions. Some analysts might have slight specificity due to the interactions with residual silanols or with active groups of attached ligands. At low flow rate the interaction of thiamethoxam and clothianidin with the adsorbent increased causing higher R_t values. The decrease of R_t of thiamethoxam and clothianidin according to increase the flow rate may be due to the presence of some excess amount of the acetonitrile or methanol close to the adsorbent surface which may cause the decrease of the insecticide interaction and also retention. It was clear that the R_t of thiamethoxam was lower than the R_t of clothianidin under all tested conditions. For example at 0.8 ml/min. with mobile phase (II), and wavelength 265 nm, the R_t values of thiamethoxam and clothianidinwere 5.46 and 7.73 min., respectively. The various analytes (pesticides) to be separated may also be arranged, based on the polarities of their functional groups.

As a general rule, in reversed-phase systems an increase in solute polarity lowers retention, due to an increased solute-eluent interaction. These findings explain the lower retention time of thiamethoxam compared to that of clothianidin. The presence of heterocyclic ring which contains in addition to the carbon atom an oxygen atom and two nitrogen atoms in thiamethoxam molecule led to an increase in its polarity than that of clothianidin.

The detector wave length of thiamethoxam as stated in the pamphlet of Syngenta Co. was 255 nm, while that of clothianidin as stated in the pamphlet of Agro Company Takeda Chemical Industries, Ltd. was 265 nm, those wave length gave maximum absorbance for each pesticide. The two wave lengths were used to detect both pesticides to choose one wave length suitable for determination of both pesticides. This was due to the presence of clothianidin in the same samples with thiamethoxam as its degradation product. The change in wave length did not change the R_t for each of them. Retention time of thiamethoxam using the mobile phase II at flow rate 0.5 ml/min under wavelength 265 and 255 nm were 9.32 and 9.12 min. The corresponding values for clothianidin were 13.14 and 13.40 min., respectively.

Effect of mobile phase:

Data show that increasing the methanol ratio from 20% to 30% resulted in increasing the area e.g. the area of thiamethoxam was 1353.4 at 0.5 ng flow rate, increased to 1872.4 with increasing the methanol to 30%.

From the table as the methanol ratio increase in the mobile phase the R_t decrease in all of the tested cases. The highest R_t obtained for thiamethoxam was 17.24 min. with methanol/ water 20/80 at flow rate 0.5ml/min.The increase of methanol to 25% (III) and to 30% (II) caused a decrease in R_t to 70% (12.13 min.) and to 54% (9.32 min.), respectively

As for the acetonitrile / 0.1%acetic acid 25/75, it was the best mobile phase under all tested conditions, (flow rates and wave length for the two tested pesticides). The areas of thiamethoxam on w.v. 255 nm under the three flow rates tested were 2398.3 for 0.5 ml/min, 1461.8 for 0.8 ml/min and 998.8 for 1.0 ml/min, which were the highest areas obtained for thiamethoxam under tested conditions.

The areas obtained for clothianidin with this same mobile phase on wavelength 265 nm were (relatively) to some extent greater than those obtained with 255 nm. But they were bigger than those obtained with the other tested mobile phases. In general the acetonitrile/acetic acid 0.1% (25/75), gave the highest clothianidin peak areas than the other three tested mobile phases.

TABLE (II)

Effect of different operating HPLC conditions on retention time (R_t), detection limit (D.L.), peak area and peak width of thiamethoxam and clothianidin

Mobile phase	Flow rate ml/min.	Thiamethoxam (10 ng)								Clothianidin (10 ng)							
		Wave length								Wave length							
		265 nm				255 nm				265 nm				255 nm			
		R_t	Area	Width	D.L.	R_t	Area	Width	D.L.	R_t	Area	Width	D.L.	R_t	Area	Width	D.L.
(I) MeOH/ Water 20 / 80	0.5	17.24	603.4	0.66	5.0	15.97	1353.4	0.77	2.5	N.D.	—	—	—	N.D.	—	—	—
	0.8	9.83	829.9	0.59	2.5	9.97	1022.5	0.57	2.5	16.18	396.8	0.61	5.0	16.23	205.8	0.48	2.5
	1.0	8.02	862.7	0.54	2.5	7.78	949.4	0.47	1.0	12.91	246.7	0.47	5.0	12.54	232.0	0.43	2.5
(II) MeOH/ Water 30 / 70	0.5	9.32	1404.8	0.65	1.0	9.12	1872.4	0.63	1.0	13.14	1000.2	0.67	5.0	13.40	642.1	0.58	2.5
	0.8	5.46	1097.9	0.44	1.0	5.46	1376.9	0.39	0.5	7.73	609.3	0.42	2.5	7.90	515.8	0.37	2.5
	1.0	4.28	825.0	0.31	0.5	4.32	1432.9	0.37	0.5	5.98	593.4	0.38	1.0	6.03	472.3	0.34	2.5
(III) MeOH/ amonium acetate 25 / 75	0.5	12.13	408.1	0.51	1.0	12.11	596.8	0.50	2.5	18.57	535.2	0.56	2.5	18.66	403.9	0.56	2.5
	0.8	7.18	359.1	0.34	1.0	7.15	514.1	0.36	1.0	10.90	526.6	0.46	1.0	10.98	442.0	0.44	1.0
	1.0	5.61	258.6	0.30	1.0	5.61	497.7	0.32	1.0	8.54	440.6	0.39	1.0	8.55	447.6	0.39	1.0
(IV)Acetonitrile /acetic acid 0.1% 25 / 75	0.5	8.06	1342.6	0.39	0.5	8.06	2398.3	0.42	0.5	10.39	1238.2	0.47	0.5	9.49	1035.3	0.43	0.5
	0.8	4.72	1082.9	0.24	0.5	4.85	1461.8	0.26	0.5	5.96	784.7	0.28	0.5	5.73	784.8	0.28	0.5
	1.0	3.85	875.9	0.21	0.5	3.85	998.8	0.18	0.5	4.68	689.0	0.23	0.5	4.69	551.9	0.23	0.5

From the Table it can be concluded that both thiamethoxam and clothianidin can be determined in the same sample, by acetonitrile/acetic acid 0.1% (25/75) with a flow rate of 0.5ml/min and under wv length 255 nm. Mobile phase is the most important parameter in the reversed-phase HPLC. Type of mobile phase used may have effect on the retention.

To discuss the obtained data about the effect of type of mobile phase used on the retention of thiamethoxam and clothianidin, Kazakevich (1996) stated that in RP-HPLC binary eluent are employed one of the eluent components is water, which does not interact with the hydrophobic adsorbent surface. And it does not compete with the analyte for the adsorption sites. Another component of any binary eluent is an active one which is called "modifier" because it can interact with the adsorbent surface and compete with analyte molecules for the adsorption sites. Increasing of the concentration of the "modifier" in the eluent leads to the decreasing of the analytes retention. Mockel *et al.* (1987a) reported that in general, retention in ODS-methanol systems is related to solute molecular surface area. Different types of surfaces produce different amounts of retention (Mockel *et al.,* 1987b). Broadly speaking, to each type of surface has to be attributed a certain polarity (Mockel *et al.*, 1987c). With pure methanol as eluent, proton-free surfaces of molecules having no dipole moment, like S_8, appear to have the lowest surface polarity. The term polarity refers to various types of molecular interactions. Thus it is anticipated that the respective types of molecular surface area behave differently towards changes in eluent polarity and structure.

They added that in methanol-water mixtures containing up to 30% (v/v) water, the surface tension increases almost linearly with increasing water content. Consequently, the free energy of cavity formation is expected to increase in the same way. In reversed–phase systems, the retention of non-ionized solutes increases with increasing eluent water content. Increasing methanol in the mobile phase caused a decrease in the Rt in both tested pesticides, wavelength and flow rates e.g. R_t of thiamethoxam was 17.24 min. at 0.5 ml/min., with 20% methanol, which became 9.32 min. at the same flow rate on increasing MeOH to 30%.

Effect of U.V. rays, sunlight and temperature on stability:

From the most important factors affecting the persistence of pesticides in the environment are temperature and light. Some pesticides are unstable when exposed to light, some decompose rapidly when exposed to relatively high temperature, and both need stabilizers, to manage in the environment.

Effect of UV rays on stability of tested insecticides:

Ultra violet rays is one of the important factors affecting the persistence of pesticides in field, as photodecomposition is positively correlated to exposure period. The effect of UV on thiamethoxam and clothianidin is shown in Table (3). It is clear that thiamethoxam degraded gradually. The percent recovery was 97.79% after two hours of exposure. It reached about 50% recovery after 18 hours, then the decomposition decreased slowly from 36.5 % to 34.0 % after 24 and 30 hours of exposure, respectively. It reached 9.97% recovery after 36 hours. Clothianidin as a degradation product was present after 6 hours of thiamethoxam exposure. Its amount fluctuated throughout the exposure time, it increased until 12 hours after which it decreased then was not detectable after 36 hours of exposure.

Clothianidin on exposure to UV rays as a parent compound was relatively more persistent with a gradual degradation. The percentages recovered were 95.6, 80.91, 68.25, 50.63 and 24.5 after 12, 24, 48, 72 and 96 hours, respectively. The half life of thiamethoxam was 17.13 h. and for clothianidin was 77.71 h. Thiamethoxam is photolytically rapidly degraded (half-life ~one hour as a droplet deposition on Teflon (Yamamoto and Casida 1999)

TABLE (III)

Effect of UV-rays on the degradation of thiamethoxam and clothianidin.

Exposure Period In hours	Thiamethoxam (120 µg)				Exposure Period In hours	Clothianidin (120 µg)		
	Amount Recovered µg	% recovered	% loss	Amount of clothianidin		Amount Recovered µg	% recovered	% loss
2	117.35	97.79	2.21	---	12	114.72	95.6	4.40
6	94.4	78.7	21.3	2.25	24	97.10	80.91	19.08
12	64.475	53.7	46.27	4.36	48	81.89	68.25	31.75
18	59.95	49.95	50.04	---	72	37.47	31.22	68.77
24	43.84	36.5	63.47	2.5	96	29.42	24.5	75.48
30	40.82	34.0	66.00	1.0				
36	11.97	9.97	90.03	N D				
t½	17.13 hours				t½	77.71hours		

Effect of sunlight on stability of tested insecticides:

Results of exposing thiamethoxam and clothianidin to sunlight are presented in Table (4). In general thiamethoxam and clothianidin decomposed as the exposure period increased, but clothianidin showed more persistence when exposed to sun light. The half life time t $_{1/2}$ of thiamethoxam was 3.19 hours while that of clothianidin was 14.75 hours. The degradation of thiamethoxam was fast through the first 4 hours of exposure, which after one hour of exposure gave a recovery of 68.97%). Clothianidin appeared as a degradation product after 6 h of exposure to sunlight, with an amount about 4%, which was stable until 14 hours, then increased on expanding the exposure period to 16 and 18 hours, to 7.86 and 7.75%, respectively.

The clothianidin on exposure to sunlight was more persistent. The photodegradation was gradual. After 2 h. of exposure the recovered percent was 94.22, then degradation continued gradually for 24 hours, reaching 40.2% recovered, then 30 hours of exposure resulted in an obvious decrease in the recovered percent to 19.4.

TABLE (IV)

Effect of sunlight on the stability of thiamethoxam and clothianidin

Thiamethoxam (80µg)					Clothianidin (80 µg)		
Time in hours	Thiamethoxam amount	% recovered	Clothianidin (as degradation product)		Time in hours	Clothianidin amount	Clothianidin Recovered %
			Amount	%			
1	55.18	68.97	---	---	2	75.381	94.22
2	41.87	52.33	---	---	3	66.32	82.90
4	39.10	48.87	---	---	6	54.02	67.52
6	17.82	22.27	3.48	4.35	12	49.20	61.50
8	15.07	18.83	3.30	4.13	14	43.98	54.98
10	10.67	13.33	3.20	4.00	18	37.15	46.44
12	10.76	13.45	3.20	4.00	24	32.16	40.20
14	10.29	12.86	3.33	4.16	30	15.52	19.40
16	8.40	10.50	6.29	7.86			
18	2.94	3.67	6.20	7.75			
t½	3.19				t½	14.75	

It could be concluded that sunlight is more effective than UV rays in accelerating the photodecomposition of thiamethoxam and clothianidin. On comparing the stability of thiamethoxam and clothianidin under the effect of UV and sunlight, it was clear that both pesticides were more stable on exposure to UV rays than on exposure to sunlight. At the same time clothianidin was more persistent than thiamethoxam.

Sensitivity to sunlight and its components from which is UV has its effect on the pesticides, their activity, persistence and either chemically modifying the molecular structure of the pesticide or the use of UV absorbing materials (stabilizers) in the formulations may affect the activity, biodegradability of the pesticide or may cause ecological problems to the soil and water pollution. (Rozen and Margulies 1991)

The radiation energy of the sun might be absorbed by a pesticide molecule principally at a given wavelength. The energy might increase the transitional, rotational, vibrational or electronic energy of the molecules. If enough energy was absorbed to interact with electrons of the molecules an electronically excited molecule would result. Energy might be disappeared or changed from the molecule in a number of ways, one of which is chemical reaction (Plimmer, 1970).

Effect of different temperature degrees on stability of tested insecticides:

Testing the effect of three different temperatures (40, 50 and 75°C) showed that there was slight effect of temperature on both insecticides. The percentages recovered were more than 95% after 7 days of exposure. These results of stability agreed with those of Yamamoto and Casida (1999) who stated that no decomposition was observed after storage of the active ingredient or formulations at 54°C after 2 months.

SUMMARY

Different chromatographic conditions were tested to reach the most convenient operating conditions to detect and determine the second generation neonicotinoids compounds and to detect the effect of some environmental factors on two neonicotinoid insecticides. Three different flow rates under wave lengths and four mobile phases were tested. The increase in the flow rates caused a decrease in the retention time (R_t), peak area and width. The R_t of thiamethoxam was lower than that of its degradation product, clothianidin, due to its polarity. The change of wavelength did not change R_t. Changing the mobile phase was very important parameter. Increasing methanol ratio increased the peak area and decreased R_t, wavelength and flow rates. Acetonitrile / 0.1 acetic acid (25/75), was the best mobile phase under all tested conditions (flow rates and wavelengths for the two tested pesticides). Using the same mobile phase, wavelength 255nm gave highest peak area for thiamethoxam at the three flow rates. While wavelength 265 nm gave greater areas for clothianidin.

Under effect of ultra violet (UV) rays and sunlight, it was clear that both pesticides were more stable on exposure to UV with half-life time (17.13 hours for thiamethoxam and 77.71 hours for clothianidin) than on exposure to sunlight which resulted in more degradation with 3.188 and 14.75 hours for thiamethoxam and clothianidin, respectively . At the same time clothianidin was more persistent than its parent compound. Testing the effect of three different temperatures showed that there was slight effect on both insecticides. The percentages recovered were more than 95% after 7 days of exposure.

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