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Influence of solvent and storage conditions on the stability of acaricide standard stock solutions

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Abstract

The stability of bromopropylate, coumaphos, amitraz and chlordimeform stock solutions in methanol and in *n*-hexane and the incidence of storage conditions have been studied using GC–MS. It has been found that the initial concentrations have a progressive depletion from the starting time, this fact is more important when methanolic solutions are stored at room temperature. Several degradation products have been detected and identified, their concentration has been rising during storage time. A lifetime for the stock solutions should be established on the basis of their diminishing concentration percentages in order to avoid further errors in quantitative analysis, below the limit the solutions should be changed for new ones.

Keywords: Stability studies; Storage conditions; Pesticides; Acaricides

1. Introduction

One of the first and most important steps in the application of analytical methods to pesticide residue control in food and environmental samples is the correct preparation of solutions from solid standards. Frequently, important errors in the quantitative analysis arising from inadequate solvent selection, the lability of the analytes, and storage conditions are found.

The stability and the useful life of stock pesticide solutions are subjects whose incidence is usually not very well considered in the scientific literature. To prepare pesticide solutions from solid standards some data can be found in the handbooks [1,2], or obtained from other articles not related directly with this aspect [3–6]; here we can point out a quite recent article, Ref. [7], in which the influence of the solvent used to prepare stock solutions of some pesticides is clearly shown. Nevertheless we have not found similar studies about the acaricides: bromopropylate, coumaphos, amitraz and chlordimeform. But as they are nowadays widely used in honey production, we thought that it would be interesting to study the stability of their solutions to avoid frequent errors in quantitative analysis.

In this work the stability of standard acaricide solutions prepared in methanol and in *n*-hexane was tested for 80 days, and under two storage conditions: room temperature without exposure to direct sunlight, and refrigeration at 4°C. Moreover, several breakdown products were detected during the study by a GC–MS system operated in scan-electron impact (EI) mode, and identified from their spectra. The evolution of these degradation products was also monitored.

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2. Experimental

2.1. Reagents

Chlordimeform, amitraz, bromopropylate and coumaphos chromatographic purity solid standards were purchased from Chemservice (West Chester, PA, USA). Analysis residue grade methanol and *n*-hexane were obtained from Scharlau (Barcelona, Spain).

2.2. GC–MS system

A Hewlett-Packard 5890 Series II gas chromatograph (Avondale, PA, USA), fitted with a 30 m×0.25 mm I.D., 0.25 μm 50% phenylmethylpolysiloxane column (called DB-17) from J and W Scientific (Folsom, CA, USA), was directly coupled to an Hewlett-Packard 5989A mass spectrometer. The temperature programme was as follows: initially 50°C, held for 1 min, then a 5°C min^{-1} ramp to 275°C, held for 8 min. The carrier gas (helium) pressure programme was: initially, 21 kPa, then a 682.6 kPa min^{-1} ramp to 276 kPa, held for 0.2 min, then a 682.6 kPa min^{-1} ramp to 35 kPa, and finally a 2.3 kPa min^{-1} ramp to 138 kPa, held for 5 min. Splitless injection (1 μl) was performed with an HP7673A automatic sampler at 200°C, the purge valve was on at 1 min. The operation conditions used in the mass spectrometer were as follows: transfer line, ion source and quadrupole temperatures, 280°C, 200°C and 100°C, respectively. Electron impact spectra were recorded in the 70–470 u range, with a threshold of 50 units. Electron multiplier voltage was maintained 100 units above the midmass autotune value.

2.3. Preparation and storage

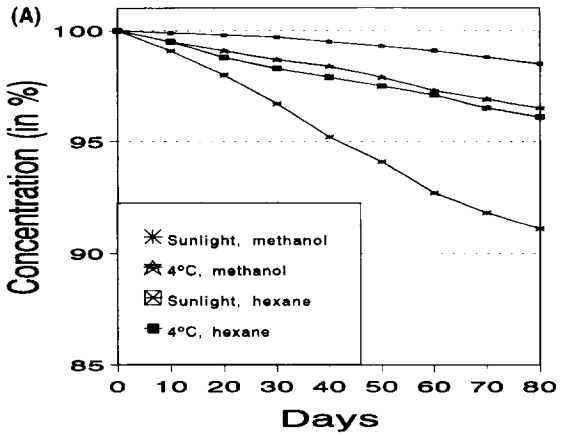
Each acaricide solid standard was solved in methanol or in *n*-hexane to achieve a final concentration of 300 mg l^{-1}. For this purpose, 75 mg of each pesticide were solved in 250 ml of solvent. Aliquots of these solutions, contained in 14 ml glass vials (screw cap septum vials from Ohio Valley Specialty Chemical, OH, USA), were kept in two different conditions: in a thermostated chamber with translucent glass doors (from Selecta, Barcelona, Spain) at 20–22°C without direct exposure to sunlight and in a refrigerator at 4°C. Three portions of each solution were regularly analyzed. The pesticide chlorpyrifos was added to the samples prior to their analysis, as a chromatographic standard, to correct the possible fluctuations in the instrument response, this pesticide solution was remade every 10 days and stored at 4°C. The identity of the degradation products was obtained with the help of spectral libraries [8,9].

3. Results and discussion

3.1. Stability in methanol

Fig. 1 shows the variation of the concentration for coumaphos (retention time, t_R, 50.17 min) and bromopropylate (t_R 44.11 min) dissolved in methanol and related to time=0. In both cases, the concentration decreased during storage, the degradation being greater in the solutions kept in room conditions. So, in the solutions kept at 4°C and 22°C, the coumaphos concentrations decreased about a 3% and 10%, and those for bromopropylate about 4% and 8%, respectively, after 80 days. The relative standard deviation for the calculated concentration was always lower than 0.5% (n=3). Degradation products of these compounds were not detected during the assay time.

Unlike the above mentioned acaricides, breakdown products related to chlordimeform were detected. Table 1 shows the most abundant mass fragments from their EI spectra and Fig. 2 shows the structures elucidated from these data. The stability of chlordimeform (t_R 29.08 min) dissolved in methanol and the occurrence of degradation products is shown in Fig. 3, reflecting on the *y*-axis the relative height of the chromatographic peaks to achieve a general idea of their evolution. The chlordimeform concentration decreased 10% at 22°C and exposure to sunlight, and about 5% in the refrigerator, for 80 days. Since the solution preparation, two persistent degradation compounds, *N*-(2-methyl-4-chlorophenyl) methoxiimine, I (t_R 20.95 min) and *N*-formyl-4-chloro-*o*-toluidine, II (t_R 29.25 min) were detected. In Fig. 4 a chromatogram with the three



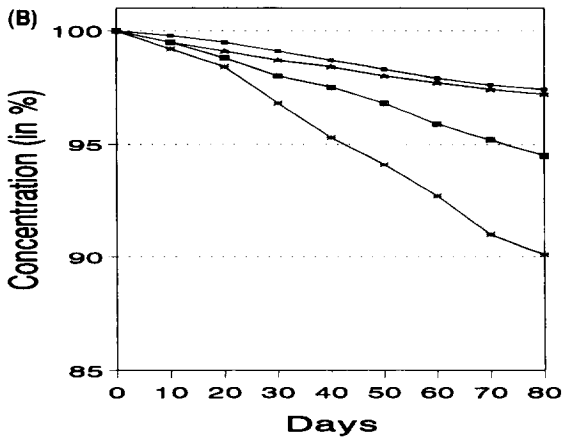


Fig. 1. Stability of bromopropylate and coumaphos dissolved in methanol and *n*-hexane. (A) Bromopropylate; (B) coumaphos.

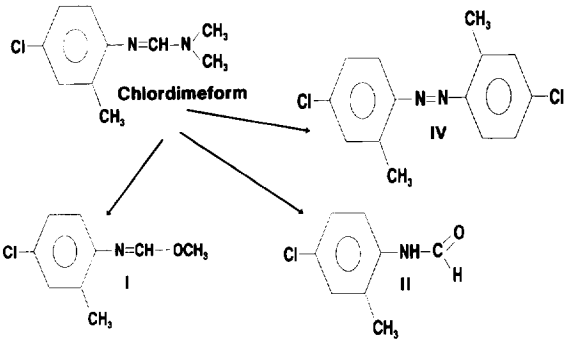


Fig. 2. Structures of the chlordimeform related compounds. See text for peak identification.

Table 1
Mass-fragments (*m/z*) and their relative abundance (in %) for the chlordimeform related compounds

Chlordimeform	Compound I	Compound II	Compound III
89 (34)	77 (40)	77 (50)	89 (49)
117 (60)	89 (35)	106 (100)	99 (12)
152 (37)	117 (85)	140 (49)	125 (100)
154 (37)	152 (100)	169 (73, M)	153 (18)
181 (66)	154 (35)	171 (24)	243 (6)
183 (21)	183 (68, M)		278 (10, M)
196 (100, M)	185 (22)		
198 (35)			

M=molecular ion.

compounds can be seen. Solutions kept long time (about 6 months) under room conditions became intense red coloured, which can be attributed to the presence of 2,2′-dimethyl 4,4′-dichloroazobenzene, III (t_R 38.16 min).

As regards amitraz, Fig. 5 shows the structures of the degradation products established from their EI mass spectra, whose most characteristic ions and their relative abundances are listed in Table 2. Fig. 6 shows the stability of amitraz (t_R 45.61 min) and the evolution of its degradation products. The amitraz degradation rate was higher in comparison with the above-mentioned acaricides. Its concentration sharply decayed in 1 day. After 10 days, the amitraz concentration was reduced by more than 90% in both storage conditions, being undetected from day 13 in both instances (detection limit 1.5 mg l^{-1}, calculated as three times the signal-to-noise ratio). In this case, the protection against the sunlight and the low temperature are not good enough to stabilize the solutions. Several degradation products were observed. The evolution of the most abundant compounds in the first days after preparing the solution, *N*-(2,4-dimethylphenyl) methoxiimine, IV (t_R 18.18 min) and *N*-(2,4-dimethylphenyl)-*N*′-methylmethanimidamide, V (t_R 27.28 min), is also given in Fig. 6. Fig. 7 shows a chromatogram obtained from an 1 day-solution. After 30 days and in both storage conditions, the main degradation products were 2,4-dimethylanaline, VI (t_R 16.54 min) and *N*-(2,4-dimethylphenyl) formamide, VII (t_R 26.33 min). Those two compounds mentioned before started to appear the third day after standard preparation, and their

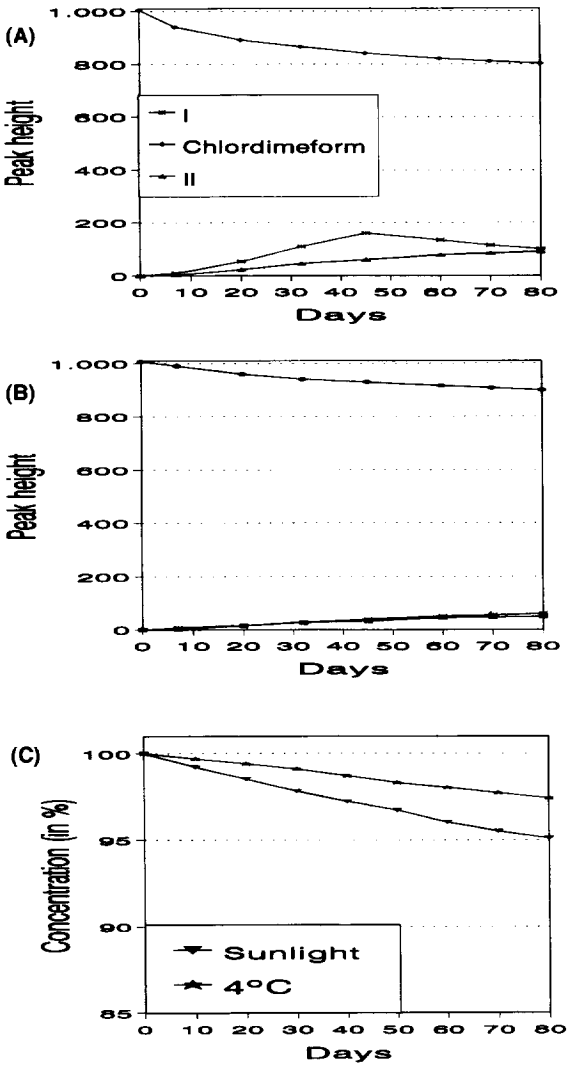


Fig. 3. Stability of chlordimeform dissolved in methanol and *n*-hexane. See text for peak identification. (A) methanol, sunlight; (B) methanol, refrigerator; (C) *n*-hexane.

concentration level was initially nearly meaningfulness in comparison with the above mentioned degradation compounds. Their occurrence increased gradually until day 30 where their peak heights were then virtually levelled off during a period of 50 days — about 350 and 700 counts for compounds VI and VII, under sunlight storage, respectively, and 250 and 600 counts for the solutions kept in the refrigerator. The occurrence of IV and V decreased from the day 20, their peak heights being very small in comparison with those for VI and VII after 30

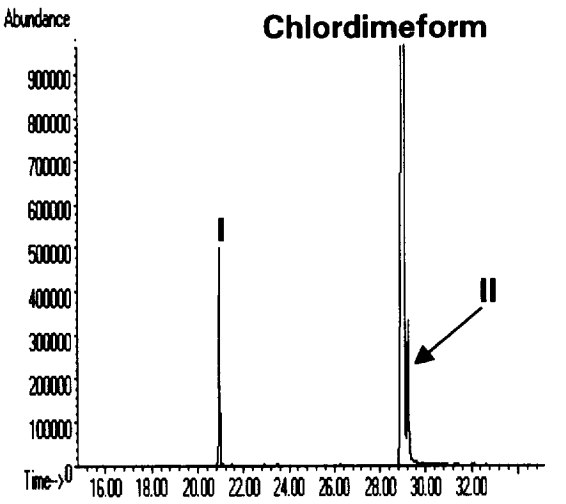


Fig. 4. Chromatogram of a chlordimeform solution in methanol. See text for peak identification.

days since the solution preparation. In Fig. 6 it can be seen that the evolution of the breakdown products is different in both storage conditions, suggesting that the breakdown products could be degraded along the amitraz.

3.2. Stability in n-hexane

Taking into account the Figs. 1 and 3 we can deduce that the stability of the standards dissolved in *n*-hexane is higher than those ones obtained in methanolic solutions. So, the concentrations obtained in solutions kept at 4°C and 22°C for 80 days have been reduced about 2% and 4% for bromopropylate,

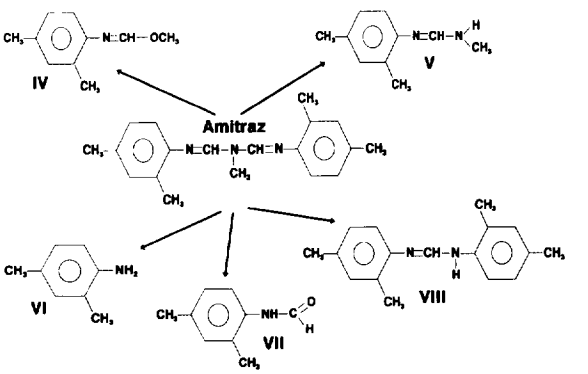


Fig. 5. Structures of the amitraz related compounds. See text for peak identification.

Table 2
Mass-fragments (*m/z*) and their relative abundance (in %) for the amitraz related compounds

Amitraz	Compound IV	Compound V
77 (19)	77 (22)	77 (38)
106 (21)	105 (14)	91 (21)
121 (80)	117 (30)	106 (55)
132 (78)	132 (100)	120 (54)
147 (73)	163 (72, M)	132 (67)
162 (100)		147 (27)
293 (65, M)		162 (100, M)
Compound VI	Compound VII	Compound VIII
77 (18)	77 (47)	77 (10)
91 (15)	91 (28)	106 (15)
106 (72)	106 (66)	121 (100)
121 (100, M)	120 (100)	132 (12)
	132 (13)	237 (3)
	149 (72, M)	252 (11, M)

M=molecular ion.

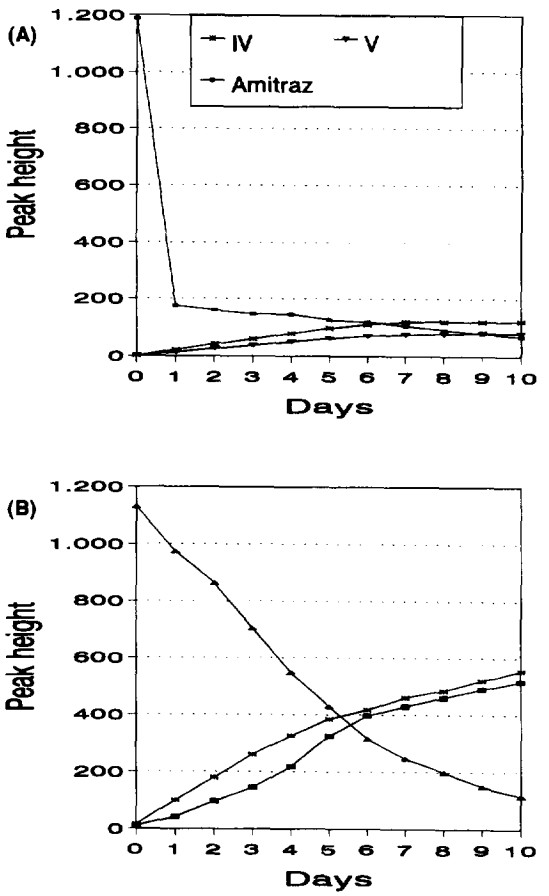


Fig. 6. Stability of amitraz dissolved in methanol. See text for peak identification. (A) Sunlight; (B) Refrigerator.

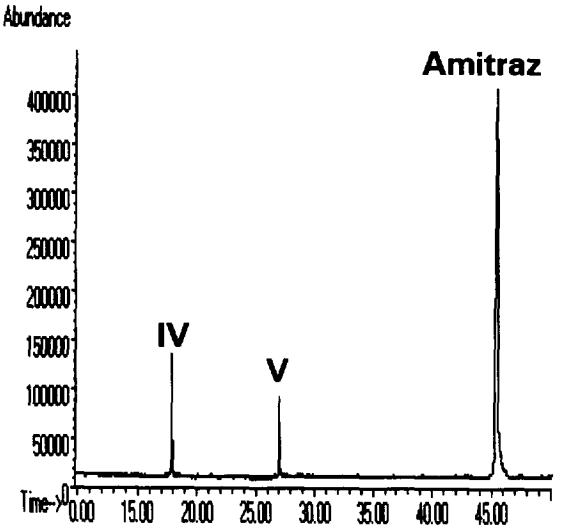


Fig. 7. Chromatogram of an amitraz solution in methanol. See text for peak identification.

3% and 5% for coumaphos and chlordimeform. In *n*-hexane solutions, degradation products were not detected during that time.

Fig. 8 shows the data obtained for the amitraz solution. The amitraz concentration decreased about 5% after keeping it for 5 days in a refrigerator and 20% at room temperature. Some degradation products were also detected but in lower proportions than in the methanolic solutions. In the sunlight exposed solution, compounds V, VII, and a new one *N,N'*-bis(2,4-dimethylphenyl) methanimidamide, VIII (t_R 41.65 min, see Table 2 and Fig. 5) were the most relevant compounds whose peak heights can be followed in Fig. 8, while VI and another unknown compound eluted at 19.62 min (*m/z* 121), were found at levels that did not exceed a peak height of 80 counts. In the refrigerated solution, compound V was the most abundant, whereas VII and VIII were found at trace levels.

4. Conclusions

The concentration of the freshly prepared acaricide solutions decreases with time as a consequence of the hydrolytic properties of the solvent and also the room conditions. Methanolic solutions of the acaricides have always lower stability than the *n*-hexane ones. The necessity of specifying the lifetime of the

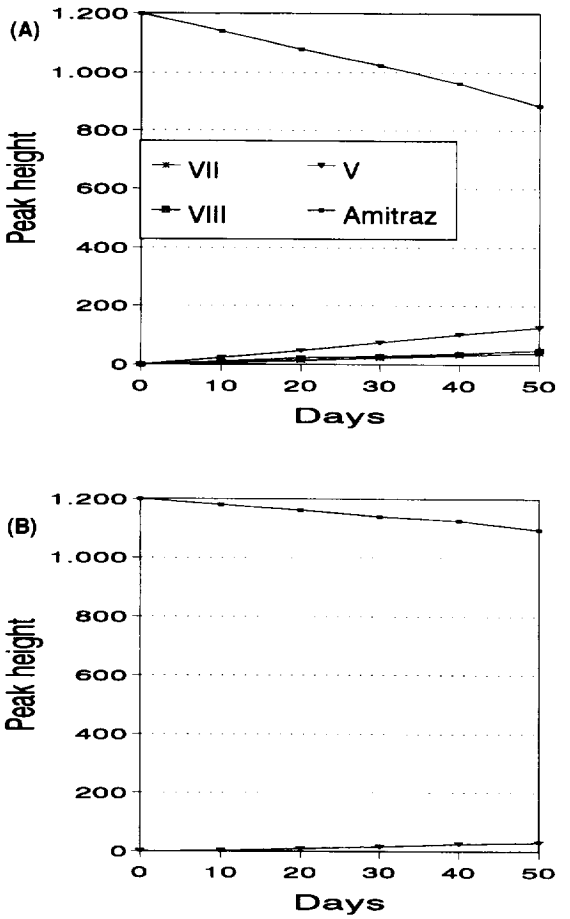


Fig. 8. Stability of an amitraz solution in *n*-hexane. See text for peak identification. (A) Sunlight; (B) Refrigerator.

stock solutions according to a tolerable error in their concentration is inferred.

To preserve the standards from degradation it is very useful to keep them at 4°C in darkness. Nevertheless, after 30 days it is advisable to remake them to prevent errors higher than 2%. The least stable solutions are the amitraz ones which begin to be degraded just after being prepared. Amitraz standard solution should be prepared in *n*-hexane just before use.

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