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Short communication

Determination of *cis* and *trans* isomers of monocrotophos in technical products by reversed-phase column liquid chromatography☆

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Abstract

A simple reversed-phase column liquid chromatographic method for the determination of *cis* and *trans* isomers of monocrotophos (MCP) using a C_{18} column, aqueous acetonitrile as eluent and UV detection at 218 nm was developed. The method was used for quality assurance and to study the relative stabilities of *cis* and *trans* isomers in technical products of MCP.

Keywords: Positional isomers; Monocrotophos; Pesticides; Organophosphorus compounds

1. Introduction

(*E*)-Dimethyl-1-methyl-3-(methylamino)-3-oxo-1-propenylphosphate [6923-24-4] widely known as *cis*-monocrotophos (*cis*-MCP) is one of the organophosphorus pesticides used as a cholinesterase inhibitor to control a broad range of pests on cotton, rice, tobacco, sorghum, sugarcane and vegetables [1]. Although it is the major constituent of the technical products of MCP, it may contain significant amounts of *trans* isomer [919-44-8] owing to the procedures involved in its manufacture [2]. These two isomers not only differ in their toxicities but also storage stabilities, therefore it is quite important to know their relative proportions in technical products [3].

A number of methods for the determination of MCP have been reported in the literature [4–7]. However, none of these methods has taken into consideration the separation of all the impurities including the isomers that are likely to be present in technical products of MCP. A large number of these methods have now been replaced by gas chromatography [8,9]. However, its value is questionable as the partition between the two energy states represented by the isomers depends on the temperature used for separation [10,11]. Liquid chromatography (LC) at room temperature seems to be a more reliable technique for measuring the ratio of isomers in a mixture.

In the present paper, a simple reversed-phase LC method for the determination of *cis* and *trans* isomers of MCP and its process related impurities using a C_{18} bonded silica with water–acetonitrile

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(87:13, v/v) as eluent and UV detection at 218 nm at ambient temperature is described.

2. Experimental

2.1. Materials and reagents

All reagents were of analytical-reagent grade unless stated otherwise. HPLC-grade acetonitrile (ACN) was obtained from Spectrochem (Mumbai, India) and technical-grade samples of MCP were obtained from United Phosphorus (Ankleshwar, India). Monomethylacetoacetamide (MMA), its chloride (MMAcl), dimer (DMMA) and trimethyl phosphite (TMP) were synthesized in our laboratory. Hydroxy *N*-methyl crotonamide (HMC) was isolated from the decomposed products of MCP and used after its identification by mass spectrometry (Micromass 70-70H, Manchester, UK). Photoisomerisation of MCP was done by exposing aqueous solutions to UV light at 254 nm using a Raynot photochemical reactor.

2.2. Apparatus

A liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a 20-μl loop injector and a high-pressure six-port valve was used. A Shimadzu SPD-6AV variable-wavelength UV–Vis spectrophotometric detector was connected after the column. A 10 μm LiChrosorb C_{18} (Merck, Darmstadt, Germany) column (250 mm×4.0 mm I.D.) was used. The chromatograms and the integrated data were recorded with a Chromatopac C-R3A processing system.

Table 1
Effect of eluent composition on capacity (k^1) and resolution factors (R_s) of *cis*- and *trans*-MCP

Composition of eluent, water–ACN (%, v/v)	k^1		R_s
	Cis	*Trans*	
97:3	19.3	9.23	3.7
92:8	14.6	6.99	3.2
87:13	6.70	3.80	2.6
82:18	3.80	2.60	0.9
77:23	3.40	2.45	0.6
72:28	2.61	2.07	0.3
67:33	2.56	2.11	0.5

2.3. Chromatographic conditions

The eluent was water–acetonitrile (87:13, v/v). Samples were dissolved in small volumes of acetonitrile and diluted with eluent. Analyses were carried out under isocratic conditions at a flow-rate of 1.0 ml/min and a chart speed of 5 mm/min at 27°C. Chromatograms were recorded at 218 nm.

2.4. Analytical procedure

Samples of MCP (≅100 mg) were dissolved in the eluent (25 ml) and 1 ml of each stock solution was diluted to 10 ml and a 20-μl volume was injected and analyzed under the above conditions. Synthetic mixtures and technical products were analyzed under identical conditions. The quantities of *cis*- and *trans*-MCP were calculated from the peak areas.

Table 2
Retention and detector response data

Compound	Code	Retention time (t_R, min)	Relative response factor	Limit of detection[a] (ng)	Absorption maximum (λ_{max}, nm)
Hydroxy *N*-methyl crotonamide	HMC	1.49	1.66	5	222
Monomethylacetoacetamide	MMA	2.85	1.67	7	217
Monomethylacetoacetamide chloride	MMAcl	3.76	1.32	9	267
trans-Monocrotophos	*trans*-MCP	4.74	1.87	5	218
cis-Monocrotophos	*cis*-MCP	7.87	1.82	5	217
Trimethyl phosphite	TMP	1.95	1.00	7	208
Dimer of monomethylacetoacetamide	DMMA	8.18	1.19	10	309

[a] $S/N=4$.

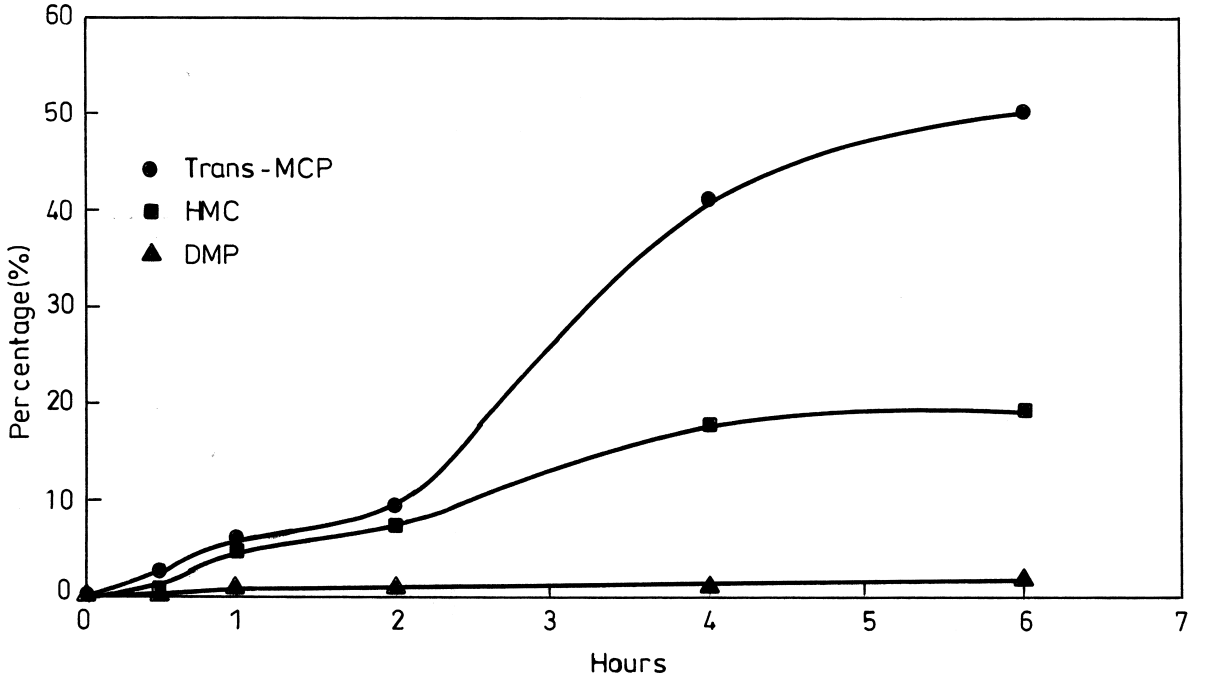

Fig. 1. Relative percentages of the products formed during the course of irradiation of *cis*-MCP with light.

3. Results and discussion

The retention data of Table 1 show that the eluent composition had a notable influence on the retention characteristics of *cis*- and *trans*-MCP. The wavelengths of maximum absorption, retention times and relative response factors at 218 nm of all compounds are given in Table 2. The wavelength of 218 nm was used for detection and quantification because all compounds except MMAcl and DMMA have the λ_{max} at 218±10 nm. The limits of detection are also presented in Table 2. Five concentrations for

Table 3
HPLC determination of *cis*, *trans* isomers and other impurities in technical products of MCP

Sample No.	MCP (%, w/w)		MMA	MMACl	HMC
	Cis	*Trans*	(%, w/w)	(%, w/w)	(%, w/w)
1	73.07	1.93	0.53	0.49	1.71
2	72.00	0.11	0.41	0.27	0.59
3	69.03	5.07	0.38	0.20	0.64
4	71.80	3.40	0.39	0.20	0.54
5	72.00	4.65	0.37	0.17	0.52
6	68.50	7.00	0.27	0.10	0.52
7	69.50	7.70	0.32	0.15	0.53
8	66.80	8.20	0.25	0.10	0.47
9	69.03	5.00	0.08	0.02	0.54
10	69.60	3.00	0.08	0.43	0.52
11	71.10	5.10	0.34	0.20	0.71
12	69.32	5.40	0.27	0.33	0.77
Range	65–75	0.1–9.0	0.05–2.0	0.01–1.0	0.1–5.0

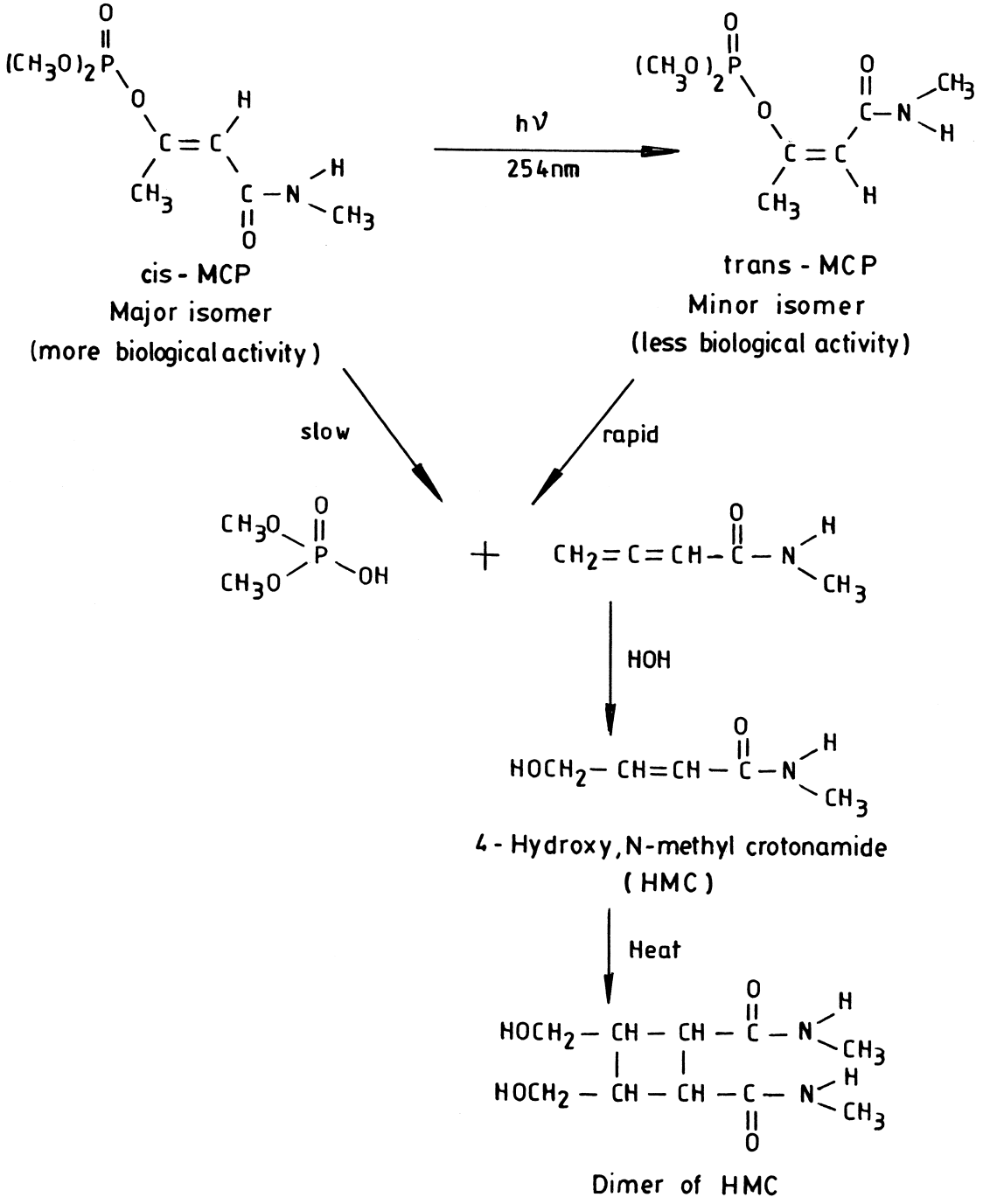

Fig. 2. Isomers of MCP and its decomposition.

impurities as well as the active ingredient in the concentration range of 0.001–0.05 mg/ml and 1.0–5.0 mg/ml, respectively, were analyzed thrice at each concentration. Excellent linearities between the amounts taken and found by LC were observed with regression coefficients greater than 0.9950.

An aqueous solution containing 5 mg/ml of *cis*-MCP was placed in a chamber containing UV lamps of 90 W and exposed to light continuously. Its conversion to *trans*-MCP was monitored by LC. The quantity of *trans*-MCP formed after 6 h of exposure was found to be around 50%. Although the concentration of the solution exposed to UV-light is quite high, it has been tested to determine the effect of light on the stability of the stock solution of MCP used during development of the present method. The levels of *trans*-MCP along with the other products of degradation formed at different intervals of time are shown in Fig. 1. The results clearly indicate that the effect of light on the assay of MCP is significant and it is necessary that the aqueous solutions of MCP are protected from light to restrict photo isomerization and possible degradation.

The *cis* and *trans* contents of MCP of the samples stored for different periods of time were calculated and their stability studied by LC. The contents of *cis*- and *trans*-MCP were found to decrease from day 0 to 30 from 72.5 to 71.7±0.4% RSD and 6.9 to 3.9±1.58% RSD, respectively. These results clearly indicate that the rates of decomposition of *cis*- and *trans*-MCP are different. On decomposition both compounds yielded a product which was identified as 4-hydroxy *N*-methylcrotonamide (HMC) by mass spectrometry. A small quantity of the same has been collected and used as one of the analytes in the present investigation. The mechanism by which it is probably formed on decomposition of *cis*-MCP is shown in Fig. 2. This scheme has been proposed by combining the results obtained in the present investigation and those available in the literature.

The applicability of the developed LC method was assessed by analyzing 10 technical MCP products received from different pesticide manufacturing industries. It was found that, in all products MCP consisted of a mixture of *cis* and *trans* isomers of which the *cis* form is the major active ingredient. The chromatogram of a typical sample of technical MCP is shown in Fig. 3 which shows that all the coexisting impurities of MCP are well separated. The results which are summarized in Table 3 show that the present LC method can be used to monitor both the *cis* and *trans* isomers and the related impurities of technical MCP products.

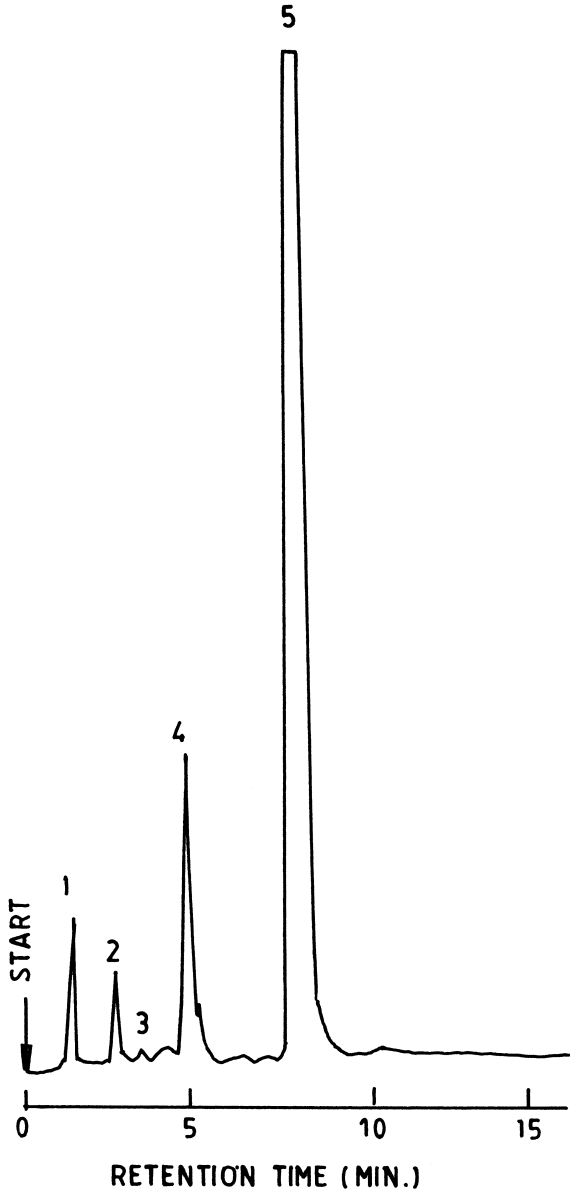

Fig. 3. Chromatograms of a technical product of MCP. Peaks: 1) HMC, 2) MMA, 3) MMAcl, 4) *trans*-MCP and 5) *cis*-MCP. For conditions see text.

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References

[1] C.D.S. Tomlin (Ed.), The Pesticide Manual, 11th ed., British Crop Protection Council, UK, 1997, p. 849.
[2] T.A. Unger, in: Pesticide Synthesis Handbook, Noyes, NJ, 1996, p. 299.
[3] E. Neuenschwander, Rev. Environ. Contam. Toxicol. 139 (1994) 41.
[4] P.B. Vitthal, S.S. Muralidhar, Talanta 41 (1994) 2127.
[5] L. Falah, W.E. Hammers, Toxicol. Environ. Chem. 42 (1994) 35.
[6] M. Subbalaxmamma, S.J. Reddy, Electroanalysis 6 (1994) 521.
[7] Y. Jinua, C. Jinhong, Fenxi Huaxue 13 (1985) 640.
[8] F.S. John, V.J. Haward, J. Assoc. Off. Anal. Chem. 70 (1987) 1014.
[9] F. Sicbaldi, A. Sarra, D. Mutti, P.F. Bo, J. Chromatogr. A 765 (1997) 13.
[10] T.J. William, Int. J. Environ. Anal. Chem. 21 (1985) 171.
[11] H.J. Stan, D. Morwetz, J. Chromatogr. 279 (1983) 173.