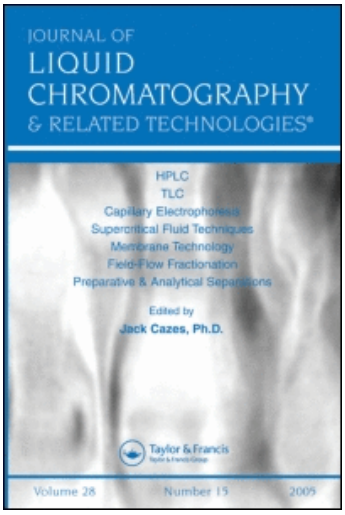

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LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF THIOPHANATE-METHYL IN TECHNICAL CONCENTRATES AND FORMULATED PRODUCTS. COMPARISON WITH THE CIPAC METHOD

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ABSTRACT

An HPLC method for the analysis of thiophanate-methyl in technical concentrates and formulated products has been developped and a comparison with the respective CIPAC method has been made. The new method uses the same elements and reagents as the CIPAC one but a narrow bore column and a diode array detector are used instead of a normal column and single wavelength detector. Precision, accuracy and resolution are very similar for both methods but the new one gives more information on the integration process, informs about the purity of peaks and causes a great saving in operating costs. Figures and data obtained by the two methods are presented.

INTRODUCTION

Nippon Soda Co. Ltd. developped and HPLC method for the analysis of thiophanate-methyl in technical concentrates and formulated products which was adopted as a full CIPAC method (1) in 1986. The use of a single wavelength UV detector and a stainless steel column 250 x 4.6 mm, packed with 10 um diameter

particles is prescribed in this method. Most control laboratories, for HPLC, use this kind of standard analytical columns.

Scott and Kucera (2,3) demonstrated that the overall sensitivity of HPLC analysis could be improved by the use of columns with reduced internal diameter, smaller particle size, and lower flow rate. Techniques to demonstrate the purity of the eluted chromatographic peaks and shorter and cheaper analytical methods are also desirable.

For these reasons, the author compares the CIPAC method for thiophanate-methyl with an HPLC method developped in his laboratory using a narrow bore column, a diode array detector, and a DPU multichannel integrator, to check the advantages of these new techniques in the analysis of pesticides.

EXPERIMENTAL

Apparatus

Millipore filters (Millipore Corp. Bedford, MA) type HAWP for water, EHWP for methanol, and FHLP for acetonitrile, pore size 0.5 um.

a) for the CIPAC method (C.M.):

High performance liquid chromatograph Hewlett-Packard 1080, equipped with a 12 uL spectrophotometer cell, microprocessor, electronic integrator, variable wavelength detector (190-600 mm), automatic variable volume injector, and recorder and computer in a single apparatus 79850B-HP LC Terminal.

b) for the new method (N.M.):

High performance liquid chromatograph Hewlett-Packard 1090, equipped with a 4.5. uL spectrophotometer cell, HP-85 personal computing system, HP-7470A graphics plotter, HP-Think-Jet printer, HP-9121 discs unit, automatic variable volume injector, diode array detector and DPU multichannel integrator.

Reagents

Eluents: for the C.M.: Acetonitrile/Methanol/Water (25/25/50) for the N.M.: Acetonitrile/Methanol (v/v)-55; Water-45.

The Acetonitrile, Methanol and Water used in this work were HPLC quality.

1) Thiophanate-Methyl, analytical standard of known purity (Nippon Soda Co. Ltd. Japan).

2) Propyl 4-hydroxybenzoate, pure, internal standard.

3) Internal standard solution: Weigh about 125 mg of propyl-4-hydroxybenzoate into a 250 mL volumetric flask, dissolve and dilute to volume with methanol.

4) Calibration solution: Weigh (to the nearest 0.1 mg) about 100 mg thiophanate-methyl analytical standard into a 200 mL volumetric flask, add methanol (150 mL) and place in an ultrasonic bath for 10 min. Allow to cool to room temperature and dilute to volume with methanol. Transfer 5.0 mL of this solution into a 50 mL volumetric flask, add 5.0 mL of internal standard solution and dilute to volume with the eluent. Filter through appropriate Millipore filter into small vial and cap.

5) Technical concentrate and wettable powder solutions:Weigh (to the nearest 0.1 mg) enough quantity of sample to contain about 100 mg thiophanate-methyl into a 200 mL volumetric flask, add methanol (150 mL) and proceed as described in 4).

6) Suspension concentrate solution: Weigh (to the nearest 0.1 mg) enough quantity of sample to contain about 100 mg thiophanate-methyl into a 200 mL volumetric flask. Add water (20 mL) and swirl to disperse the sample. Add methanol (150 mL) and proceed as described in 4).

7) Dustable powder solution: Weigh (to the nearest 0.1 mg) enough quantity of sample to contain about 50 mg thiophanate-methyl into a 200 mL volumetric flask, add methanol (150 mL) and place in an ultrasonic bath for 10 min. Allow to cool to room temperature and dilute to volume with methanol. Transfer 10.0 mL of the clear supernatant solution into a 50 mL volumetric flask, add 5.0 mL of internal standard solution and dilute to volume with the eluent. Filter through appropriate Millipore filter into small vial and cap.

TABLE 1

Chromatographic Conditions for Both Methods

	C.M.	N.M.
Stainless Steel Column	250 x 4.6 mm	100 x 2.1 mm
Stationary phase	RP-8 on Lichrosorb 10 µm	ODS-Hypersil 5 µm
Eluent	$CH_3CN/CH_3OH/H_2O$: (25-25-50)	CH_3CN/CH_3OH (v/v)-55 H_2O 45
Flow rate	1 mL/min	0.3 mL/min
Stop time	9 min	2.5 min
Injected Volume	20 µL	2 µL
Detector Wavelength Setting	269 nm vs 430 nm	A:269,4-550,100 nm B:254,4-550,100 nm
Temperature	40ºC	40ºC
Chart speed	0,5 cm/min	2 cm/min
Attenuation	2^7	automatic

Chromatographic conditions:

Table 1 gives the chromatographic conditions for both methods: C.M. (CIPAC method) and N.M. (new method).

Calibration and quantitation

Inject the determined injection volume of standard,for every method, into the appropriate chromatograph until variation in standard peak areas is less than 1%. Adjust detector sensitivity in order to obtain peak heights ca. 60-80 % full scale for the 1080 apparatus and fix automatic range for the 1090 one. Calibrate and inject the determined injection volumes of the samples to be analyzed. Concentrations are proportional to areas at the levels established in this paper.

RESULTS AND DISCUSSION

Table 2 shows the comparison of the results obtained, in g Kg^{-1}, in the analyses of a Technical Concentrate, a Wettable Powder, a Suspension Concentrate, and a Dustable Powder of thiophanate-methyl, both by the CIPAC (C.M.) and the new (N.M.) method. The standard relative deviation (S_r) goes from 1.24 to

TABLE 2

Thiophanate-Methyl Content in g Kg^{-1} by Both Methods (C.M. and N.M.)

	Technical Concentr.		Wettable Powder		Suspension Concentr.		Dustable Powder	
	C.M.	N.M.	C.M.	N.M.	C.M.	N.M.	C.M.	N.M.
	982.0	986.5	709.5	667.5	425.9	409.1	21.97	21.81
	986.6	962.6	701.8	672.5	435.0	412.3	21.94	21.18
	960.6	948.6	725.8	713.7	440.7	433.2	21.66	22.15
	985.0	955.8	716.5	712.8	448.8	432.8	22.72	22.20
	=====	=====	=====	=====	=====	=====	=====	=====
$\bar{x}$	978.6	963.4	713.4	691.6	437.6	421.9	22.07	21.84
μ*	± 19.3	± 26.2	± 16.3	± 39.9	± 15.3	± 20.6	± 0.72	± 0.75
s_r	1.24	1.71	1.43	3.62	2.20	3.07	2.06	2.15
t_{exp}	1.486		1.609		1.952		0.697	

* = confidence interval

$t_{.05,\ 6\ d.f.} = 2.447$, $t_{.01,\ 6\ d.f.} = 3.707$

TABLE 3

Test of Recovery for Thiophanate-Methyl by the CIPAC (C.M.) and the New Method (N.M.)

New Method			
Ad. THIOPH. ng/2 μL	Found THIOPH. ng/2 μL	Recovery %	S_r*
7.02	7.35 ± 0.35	104.70	1.91
14.04	13.97 ± 0.06	99.50	0.16
21.06	21.42 ± 0.33	101.71	0.62
28.08	28.41 ± 1.16	101.18	1.64
CIPAC Method			
Ad. THIOPH. ng/20 μL	Found THIOPH. ng/20 μL	Recovery %	S_r*
70.2	69.9 ± 19.2	99.57	11.03
140.4	141.3 ± 12.4	100.64	3.54
210.6	213.8 ± 13.2	101.52	2.48
280.8	295.0 ± 18.7	105.06	2.55

* Standard relative deviations of three determinations.

3.62 and, in all cases, the experimental t was inferior to the tabled value for t. So, it is concluded that the mean values by both methods are not different.

The standard addition method was used to test the accuracy of both methods. Recoveries went from 99.50 to 104.70%, with a relative standard deviation of 0.16-1.91, for the new method (N.M.) and from 99.57 to 105.06% with a relative standard deviation of 2.48-11.03, for the CIPAC method (C.M.) as shown in Table 3. The good recovery obtained in all cases means that Beer's Law, at the tested concentrations, is followed in the two methods.

Fig. 1 shows the chromatography of(a) a Thiophanate-Methyl Technical Concentrate by the CIPAC method,(b) a Thiophanate-Methyl Dustable Powder by the new method in real time and(c) the replot in reprocess of this last chromatogram with special annotation

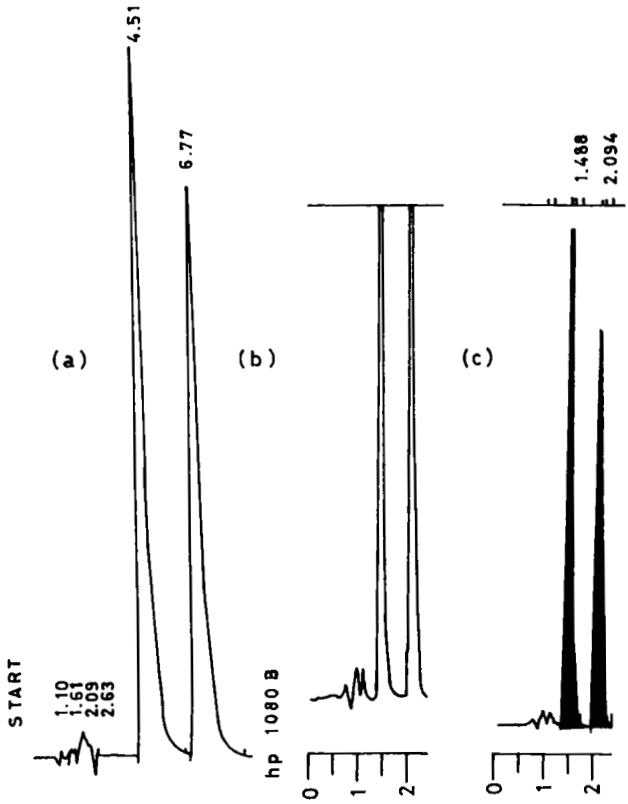

FIGURE 1: Chromatography of: (a) Thiophanate-Methyl Technical Concentrate by the CIPAC method, (b) Thiophanate-Methyl Dustable Powder by the new method, and (c) the previous chromatogram (b) with special hatched annotation.

where baseline, retention times, tick marks and hatched shading of integrated areas are shown. This is an advantage of the new method on the CIPAC one, since it informs us just how correct the integration process is. On the other hand it shows how the information obtained in a chromatographic development can be dealt with for further calculations and /or representations without repeating the chromatographic development. Separation seems to be good by both methods and resolution in(b) is at least as good as

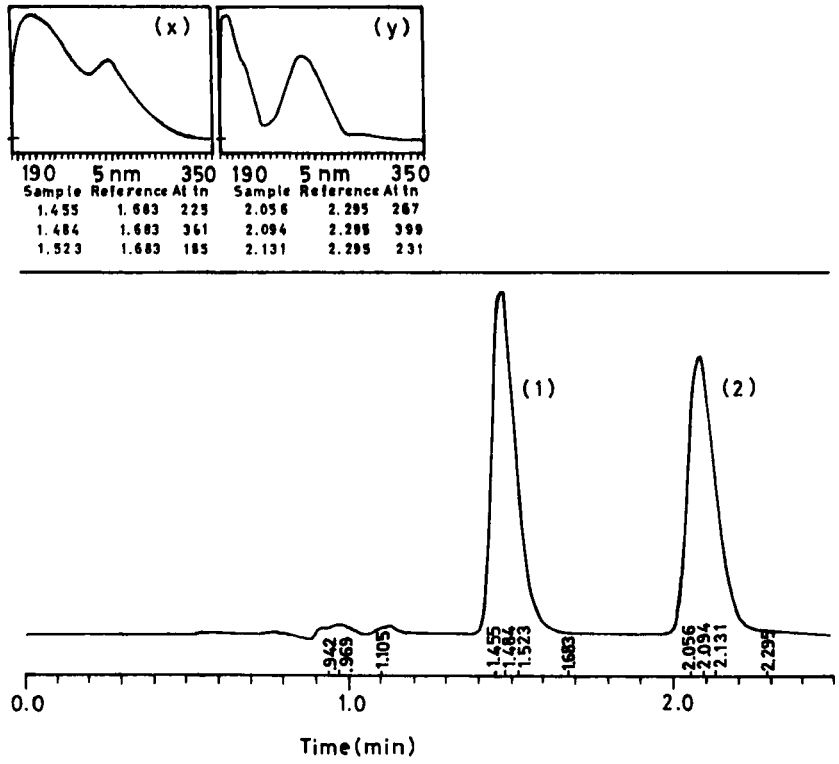

FIGURE 2: Signal plus spectra plot of the previous chromatogram (b). (1) Thiophanate-Methyl, (2) Propyl 4-hydroxybenzoate, (x) three superposed spectra obtained at different times of the chromatographic peak (1), (y) three superposed spectra obtained at different times of the chromatographic peak (2).

in(a). It must be pointed out that approximately the same chromatograms are obtained with the suspension concentrate sample and the calibration solution, although they are not given in order to be brief. The retention time of thiophanate-methyl for the sample solutions did not deviate by more than 1% from those for the calibration solution, what is taken as a confirmatory test of identity, by both methods.

Fig. 2 shows the signal plus spectra plot of the same previous chromatogram(b) and informs about the purity of the eluted chromatographic peaks. For that purpose, the detector performs three scannings at three points (times) in every chromatographic peak: Prior, at and after every maximum. If the three spectrochromatograms are similar, as it is shown on the upper left-hand side of Fig. 2, the peaks correspond to pure substances, in this case thiophanate-methyl for the first three spectra and propyl-4-hydroxybenzoate for the second three. This demostration of the purity of every chromatographic peak is only possible with the use of a diode array detector and a multichannel integrator, so,it is an advantage of the N.M. on the C.M. The identical shape of the spectra for the analytical standard and the active ingredient of the samples is a second confirmatory test of identity.

The thiophanate-methyl spectra shows a maximum of absorbance at 269 nm while the one corresponding to propyl-4-hydroxybenzoate shows a maximum of absorbance at 254 nm. Therefore these are the two chosen wavelengths for simultaneous integration. The ratio of the signals obtained at those two wavelengths is shown in Fig. 3 both for the thiophanate-methyl and the internal standard peaks of the suspension concentrate solution. The straight shape of that ratio of signals for both peaks is a second demonstration of their purity. This demonstration is another advantage of the N.M. on the C.M., due to the performances of the diode array detector and the multichannel integrator.

The advantages of this kind of detectors have been perfectly pointed out by Lazaro et al.(4).

TABLE 4

Savings in Time and Eluent.

	C.M.		N.M.
Fow rate	1 mL/min		0.3 mL/min
Run (Stop Time)	9 min		2.5 min
Saving in time		62 %	
Saving in eluent		92 %	

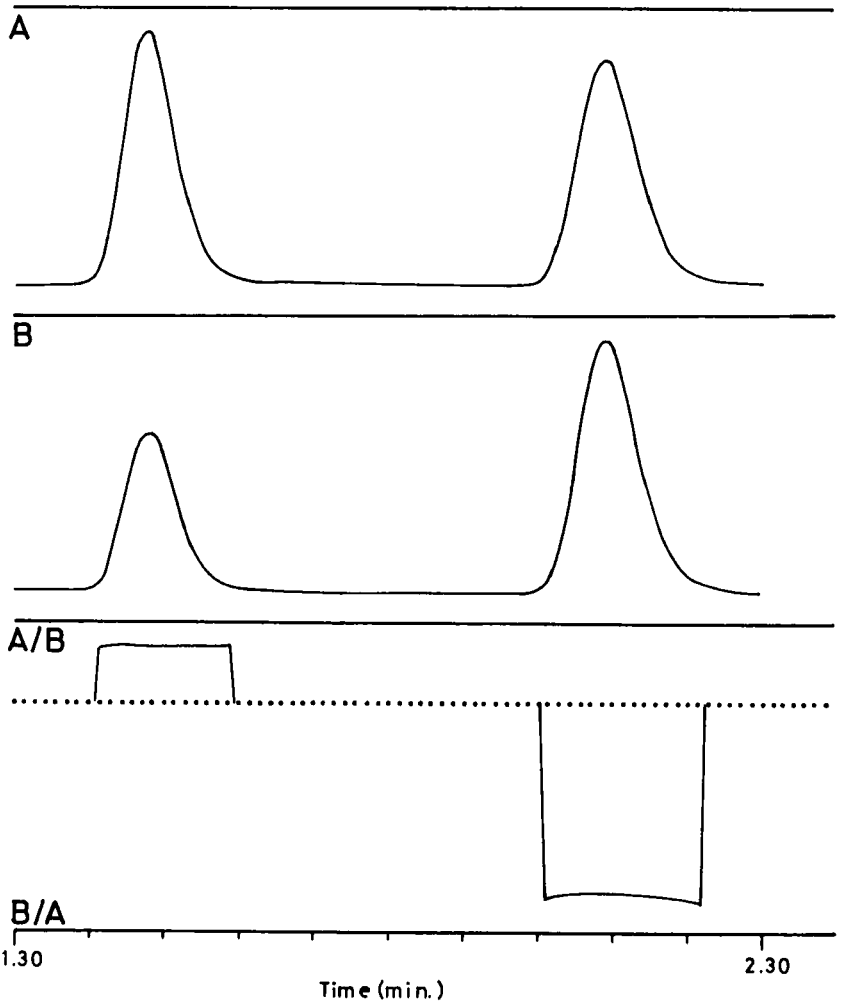

FIGURE 3: Ratio of signals of Thiophanate-Methyl and Propyl 4-hydroxybenzoate in a Suspension Concentrate sample, measured at: A(269 nm) and B(254 nm). A/B quotient of the two signals.

Table 4 shows the saving in time and eluent obtained by using the new method in comparison with the CIPAC one and that means not only a saving in eluent of up to 92% but also and mainly a saving in operating hours and operating costs which increases productivity in such a way that it provides dramatic benefits. It must be pointed out that the use of less eluent also decreases the cost of disposal.

Because the new method provides similar precision, accuracy and resolution than the CIPAC one, gives more information on the integration process, informs about the purity of peaks and causes an enormous saving in operating costs, the author suggests that liquid chromatography with narrow bore columns, diode array detectors and multichannel integrators be used preferentially by CIPAC, in its international work of normalization of analytical methods for pesticides, as soon as this kind of liquid chromatographs becomes more or less normal in most laboratories.

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