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APPENDIX

Table 1 R_f Values of Mebendazole, 2,4-DNPH and 2,4-DNPH' zone Derivative on Silica gel 60 GF 254, Chloroform as Mobile Phase

Compound	R_f Value	Color of spot
1. Mebendazole	0.19	purple (under uv-light 254 nm)
2. 2,4-dinitrophenyl- hydrazine	0.49	yellow
3. 2,4-dinitrophenyl- hydrazone derivative	0.30	orange

Table 2 Chemical Reactions of 2,4-DNPH and Other Carbonyl
Compounds in 2 N Sulphuric acid

Carbonyl Compounds	Result
1. Benzophenone	an orange precipitate occurred
2. Carbachol	(no change)
3. Mebendazole	an orange precipitate occurred
4. Methocarbamol	(no change)
5. Parbendazole	(no change)
6. Ronidazole	(no change)

Table 3 Chemical Reactions of 2,4-DNPH and Other Carbonyl Compounds in Acidified Methanol

Carbonyl Compounds	Results	Results after adding 2 N H ₂ SO ₄
1. Benzophenone	an orange precipitate occurred	an orange precipitate
2. Carbachol	(no change)	(no change)
3. Mebendazole	solution was deeper orange	an orange precipitate occurred
4. Methocarbamol	(no change)	(no change)
5. Parbendazole	(no change)	(no change)
6. Ronidazole	(no change)	(no change)

Table 4 Effect of Temperature

Temperature (°C) of Warming for 1 hr.	Absorbance ^a at 393 nm	% C.V.
room temp. (27°)	0.281 ± 0.006	2.115
50°	0.447 ± 0.003	0.739
60°	0.496 ± 0.003	0.579
70°	0.509 ± 0.004	0.808
80°	0.505 ± 0.004	0.830
100°	0.448 ± 0.030	6.784

^aMean value ± S.D. of four experiments



Table 5 Effect of Time at 70°C

Time of warming at 70°C (min)	Time of setting at room temp. (min)	Abs. ^a at 393 nm ± S.D.	% C.V.
-	120	0.278 ± 0.009	3.115
15	105	0.434 ± 0.002	0.511
30	90	0.475 ± 0.002	0.360
45	75	0.489 ± 0.004	0.835
60	60	0.489 ± 0.004	0.790
90	30	0.490 ± 0.002	0.486

^aMean value ± S.D. of four experiments

Table 6 Effect of Time for Completed Reaction at Room Temperature

Time of Warming at 70°C (min)	Time of Setting at room temp. (min)	Abs. ^a at 393 nm ± S.D.	% C.V.
60	30	0.491 ± 0.005	1.043
60	60	0.493 ± 0.003	0.606
60	90	0.497 ± 0.001	0.285
60	120	0.499 ± 0.002	0.413
60	1 day	0.510 ± 0.002	0.467

^aMean value ± S.D. of four experiments

Table 7 Effect of Acidity

Acidity of Reagent	Absorbance ^a at 393 nm \pm S.D.	% C.V.
1.5 N	0.474 \pm 0.004	0.912
2.0 N	0.476 \pm 0.004	0.947
2.5 N	0.475 \pm 0.004	0.910
3.0 N	0.460 \pm 0.009	1.965
4.0 N	0.443 \pm 0.007	1.632

^aMean value \pm S.D. of four experiments

Table 8 Optimum Ratio of Mebendazole to 2,4-DNPH

Ratio of Mebendazole:2,4-DNPH			Absorbance ^a at 393 nm \pm S.D.	% C.V.
by volume (ml)	by weight (mg)	by mole		
1 : 1	1 : 3.35	1 : 5	0.446 \pm 0.003	0.669
1 : 2	1 : 6.70	1 : 10	0.469 \pm 0.004	0.779
1 : 3	1 : 10.05	1 : 15	0.473 \pm 0.004	0.913
1 : 4	1 : 13.40	1 : 20	0.476 \pm 0.005	0.974
1 : 5	1 : 16.75	1 : 25	0.475 \pm 0.002	0.497
1 : 6	1 : 20.10	1 : 30	0.474 \pm 0.002	0.456
1 : 7	1 : 23.45	1 : 35	0.468 \pm 0.004	0.799

^a Mean value \pm S.D. of four experiments

Table 9 Effect of Time on Stability of 2,4-DNPH' zone Derivative

Time (min)	Absorbance ^a at 393 nm	% C.V.
5	0.538 ± 0.002	0.356
15	0.539 ± 0.002	0.303
30	0.540 ± 0.001	0.151
45	0.542 ± 0.002	0.315
60	0.541 ± 0.001	0.151
90	0.540 ± 0.001	0.107
120	0.542 ± 0.001	0.177
150	0.540 ± 0.001	0.185
180	0.540 ± 0.001	0.177
18 hr.	0.541 ± 0.001	0.177
24 hr.	0.541 ± 0.001	0.213

^aMean value ± S.D. of four experiments

Table 10 Linearity of Absorbance against Concentration of
Mebendazole

conc. of mebendazole mcg/ml	Absorbance ^a at 393 nm	% C.V.
1.012	0.101 ± 0.003	2.915
2.024	0.202 ± 0.001	0.623
3.036	0.304 ± 0.002	0.570
4.048	0.402 ± 0.003	0.704
5.060	0.494 ± 0.003	0.669
6.072	0.600 ± 0.004	0.750
7.084	0.694 ± 0.003	0.448

^aMean value ± S.D. of four experiments

Correlation coefficient (r) = 0.9999

Table 11 Percent Labelled Amount of Mebendazole in Mebendazole Tablets Using 2,4-DNPH Method and USP XXI Method (modified)

Manufacturer	Percent labelled amount ^a of mebendazole tablet			
	2,4-DNPH Method		USP XXI Method	
	% LA	% C.V.	% LA	% C.V.
A	107.12	0.814	107.26	2.459
B	103.09	0.516	103.30	0.907
C	100.43	0.716	101.04	0.682
D	99.16	0.779	97.28	0.679
E	97.09	0.970	97.83	0.360

^aMean value of four experiments

Table 12 Percent Recovery of Mebendazole in Mebendazole Tablets
by Using 2,4-DNPH Method and USP XXI Method (modified)

Mebendazole added (mg)	2,4-DNPH Method ^a		USP XXI Method ^a	
	% Recovery	% C.V.	% Recovery	% C.V.
10	98.60	1.688	101.44	1.554
20	100.19	1.338	99.59	1.827
30	102.67	0.620	101.42	1.850

^a Mean value of four experiments

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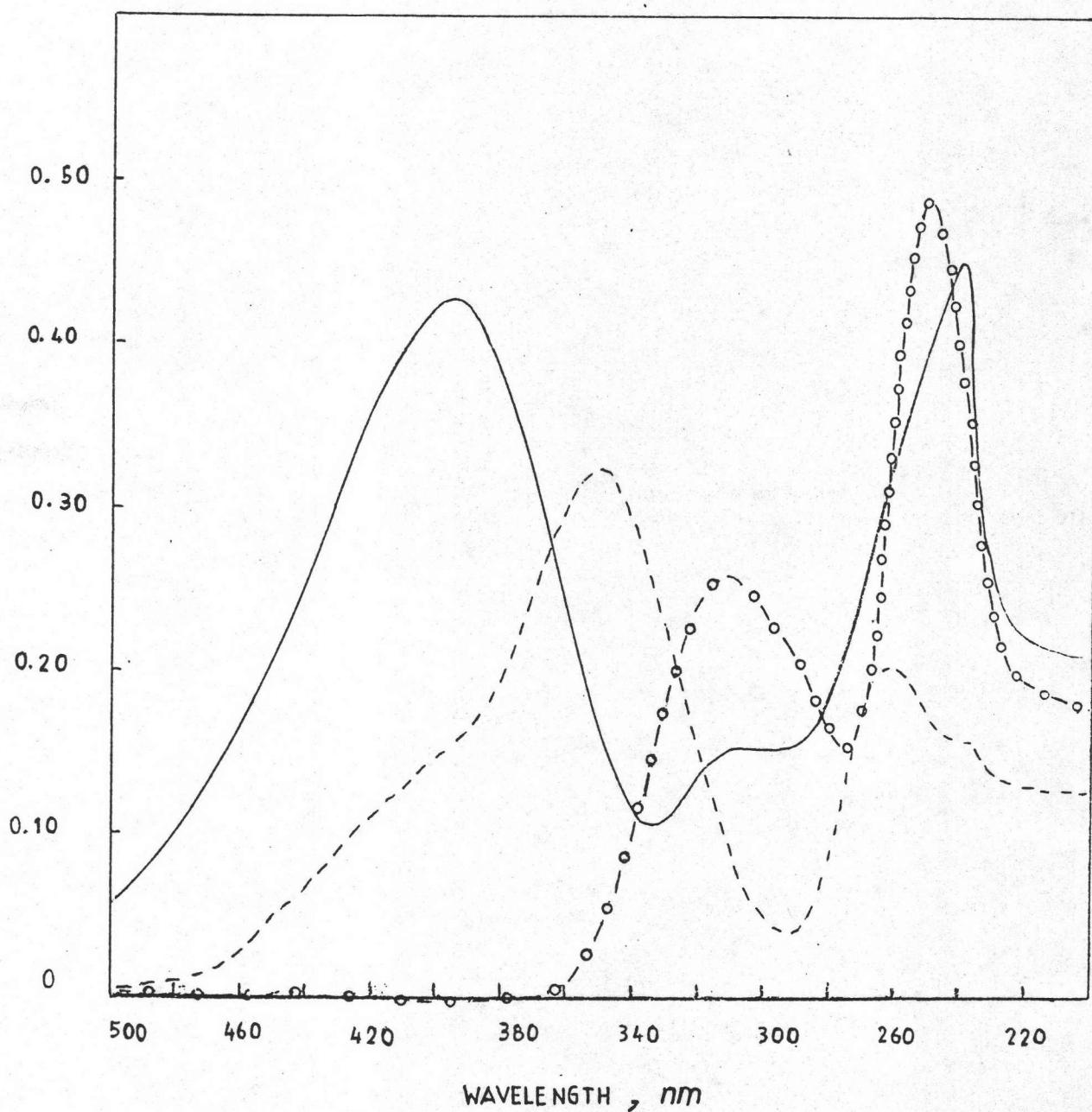


Figure 2 Absorption spectra of mebendazole, 2,4-dinitrophenylhydrazine and 2,4-dinitrophenylhydrazone

Key : —○—○—○—○ mebendazole
 ----- 2,4-dinitrophenylhydrazine
 _____ 2,4-dinitrophenylhydrazone

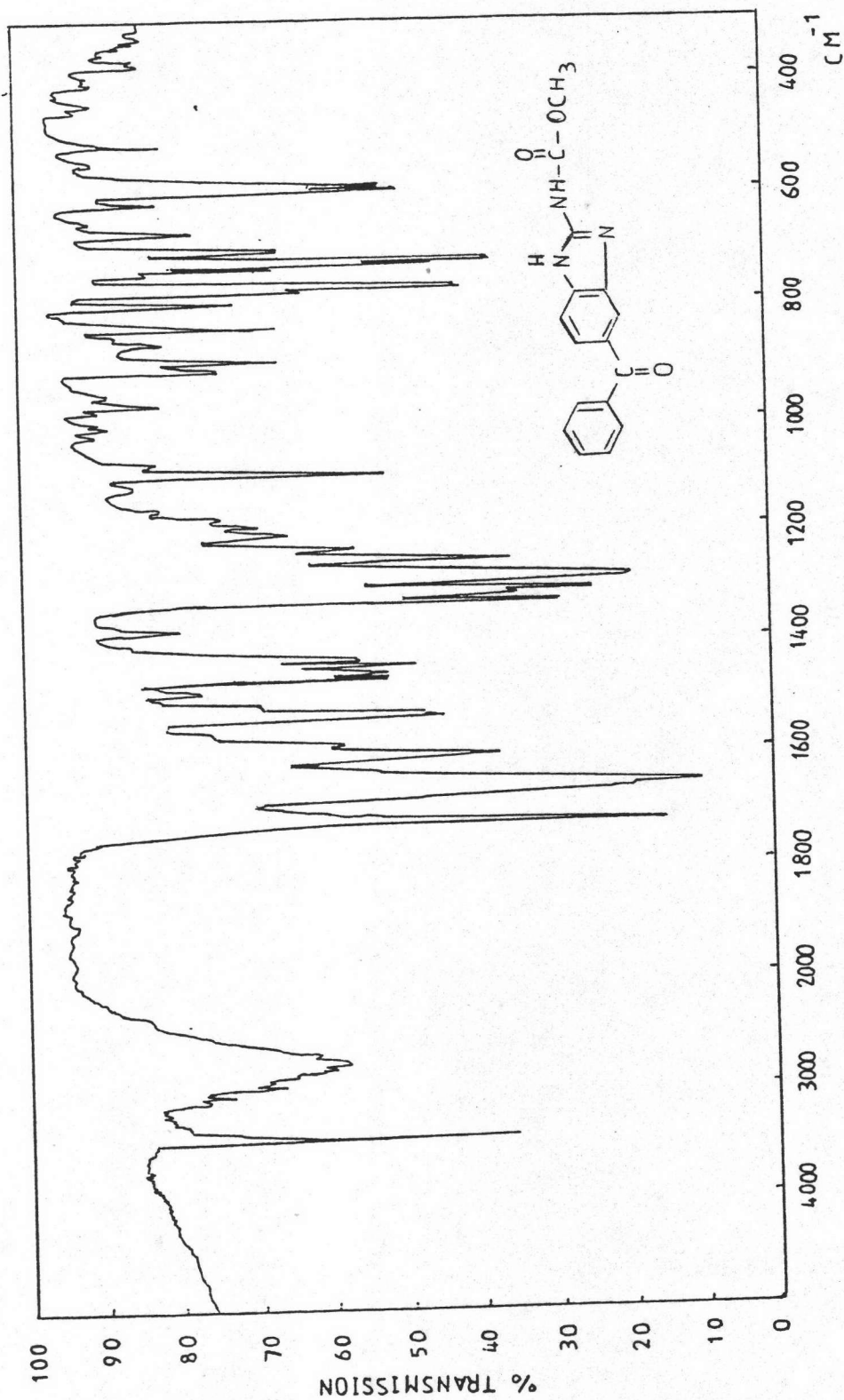


Figure 3 Infrared spectrum of mebendazole (KBr)

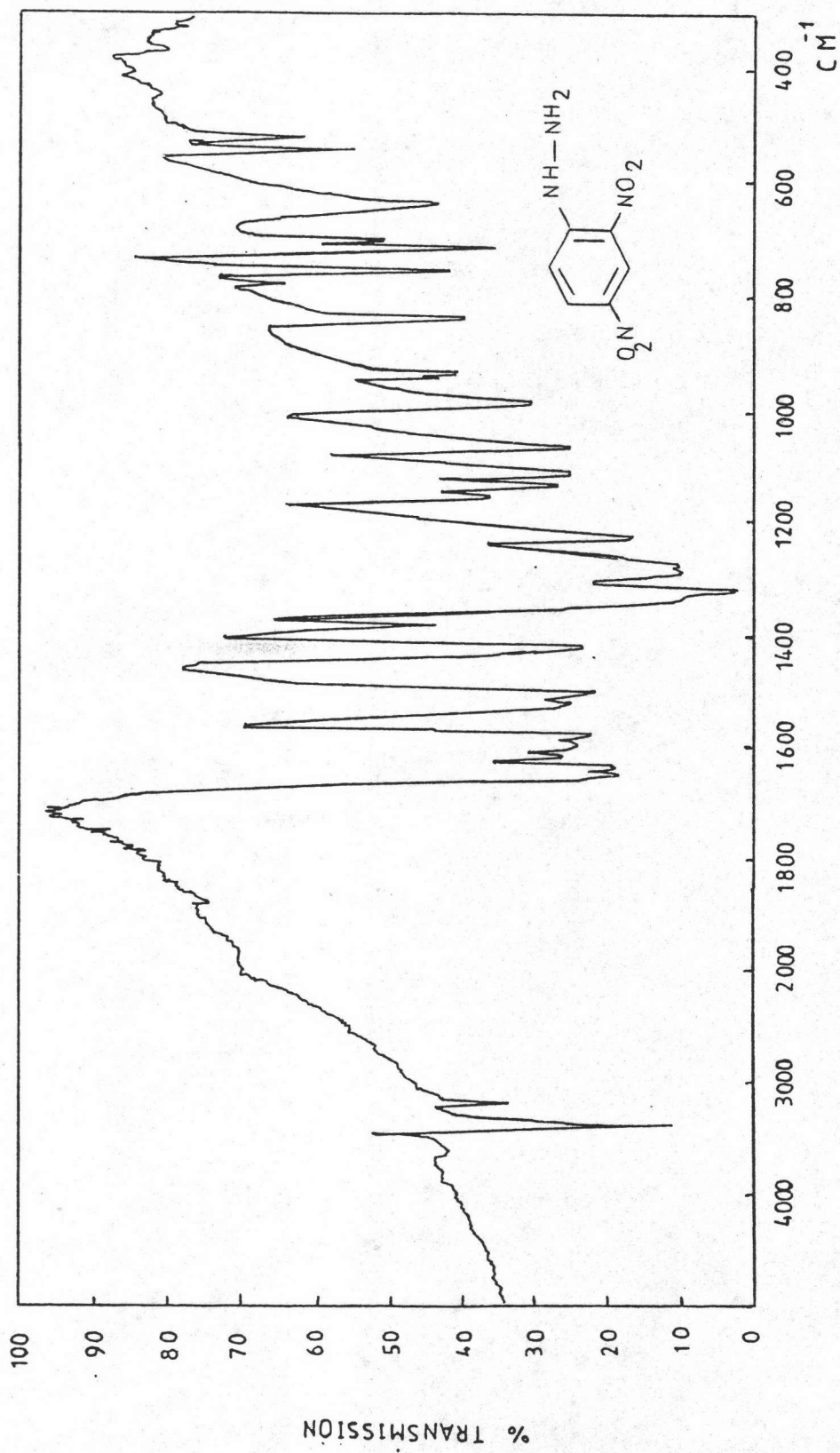


Figure 4 Infrared spectrum of 2,4-dinitrophenylhydrazine (KBr)

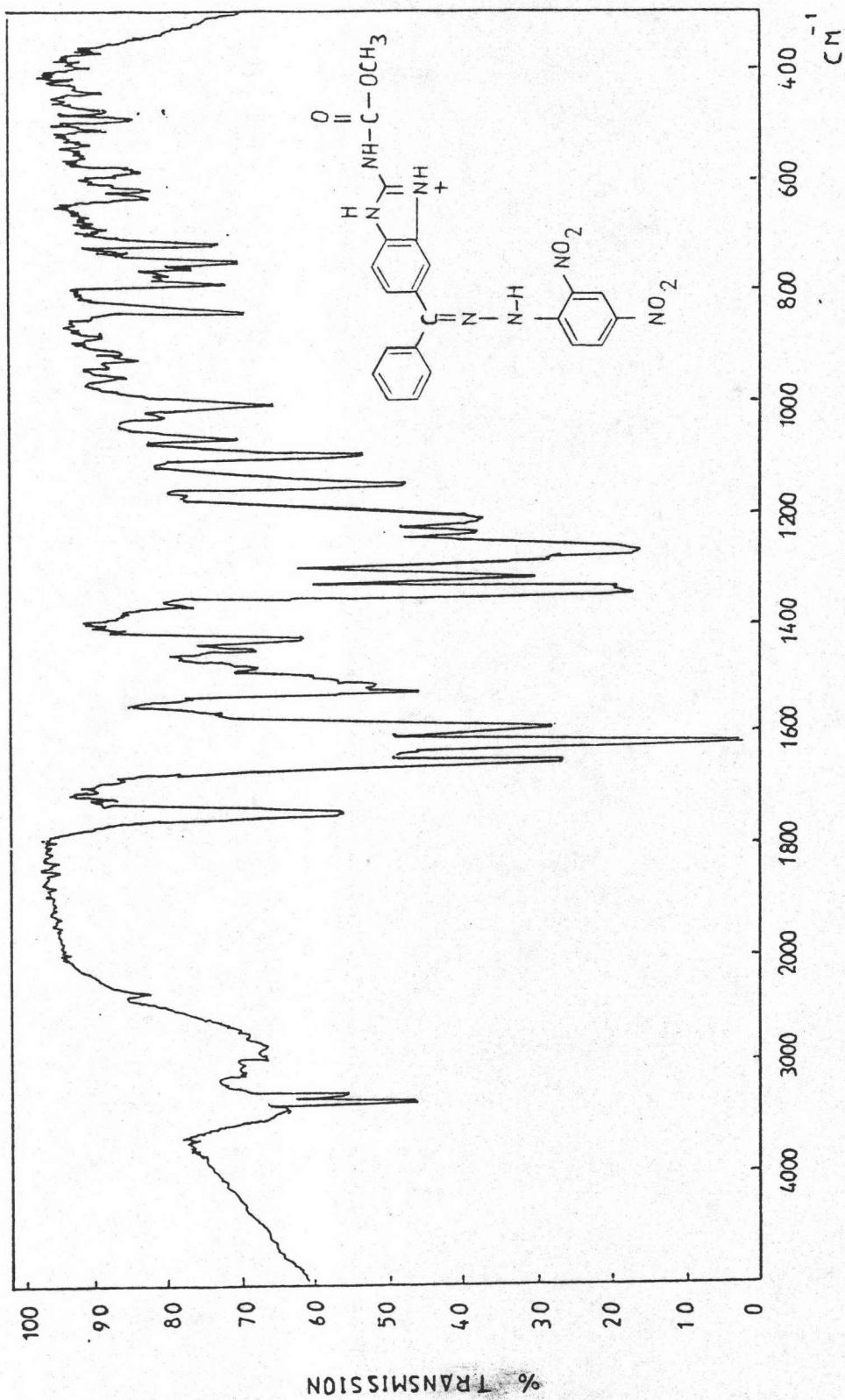
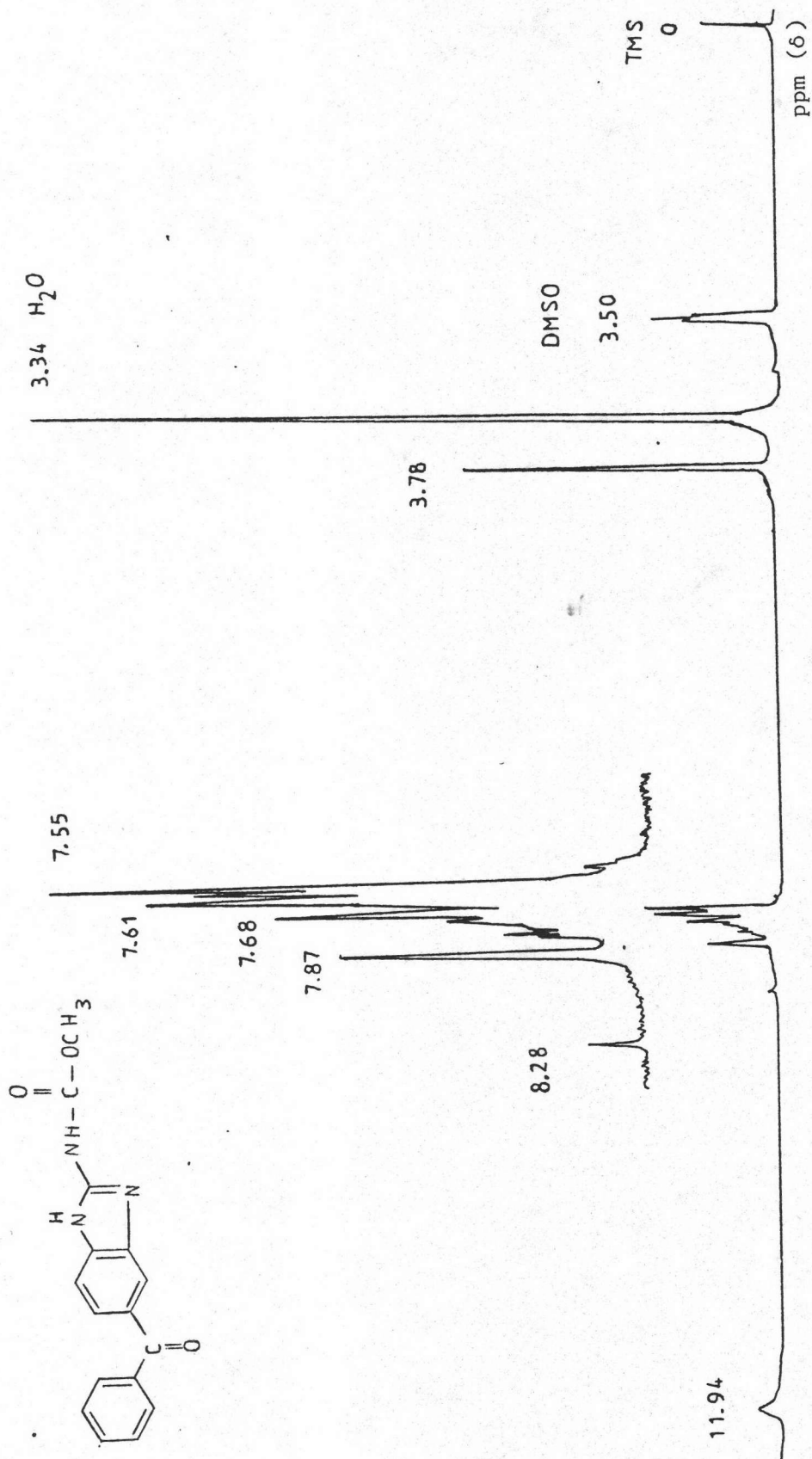


Figure 5 Infrared spectrum of 2,4-dinitrophenylhydrazone derivative (KBr)

Figure 6 ^1H -nuclear magnetic resonance spectrum of mebendazole in DMSO-d_6

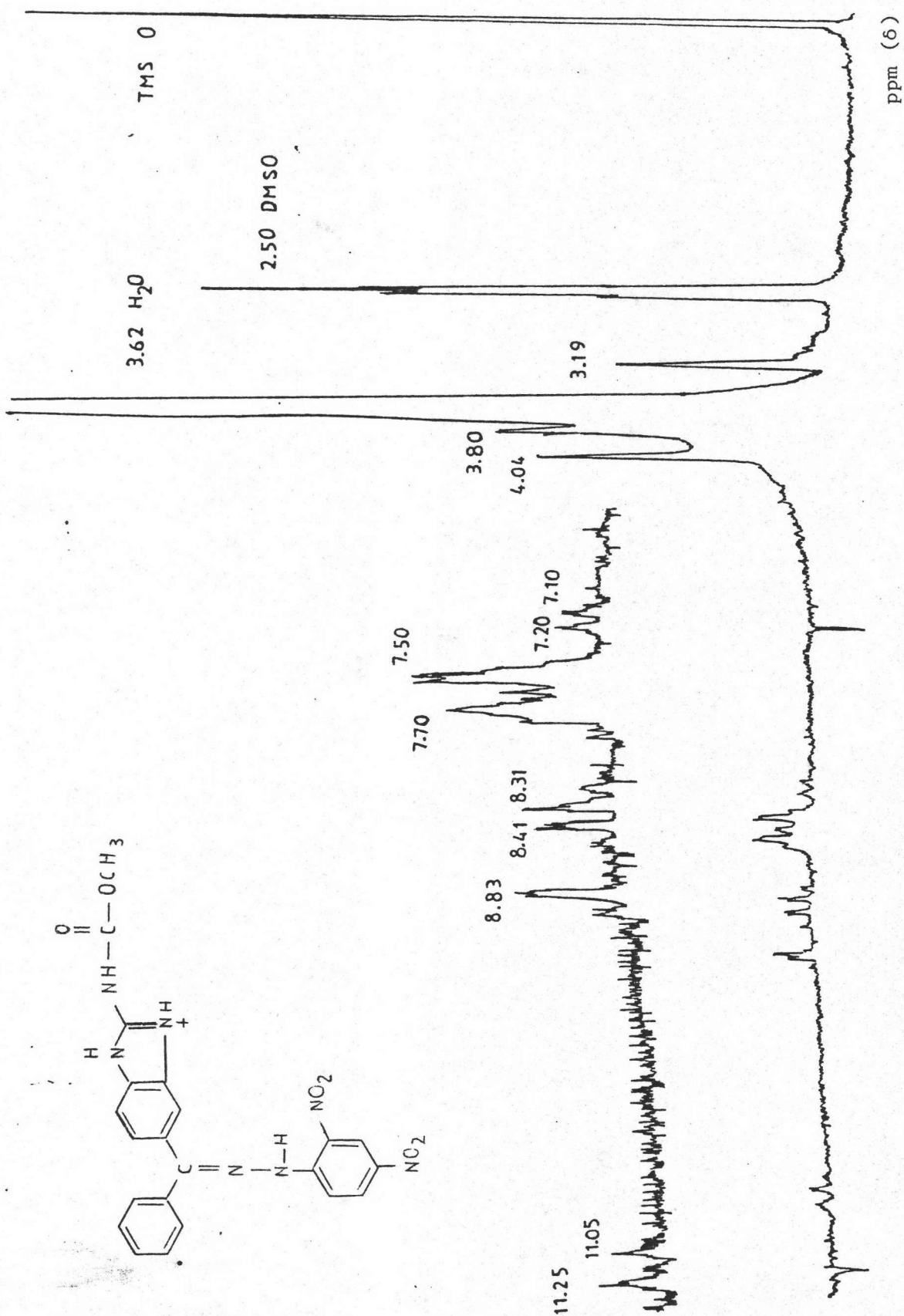


Figure 7 ¹H-nuclear magnetic resonance spectrum of 2,4-dinitrophenylhydrazone in DMSO-d₆

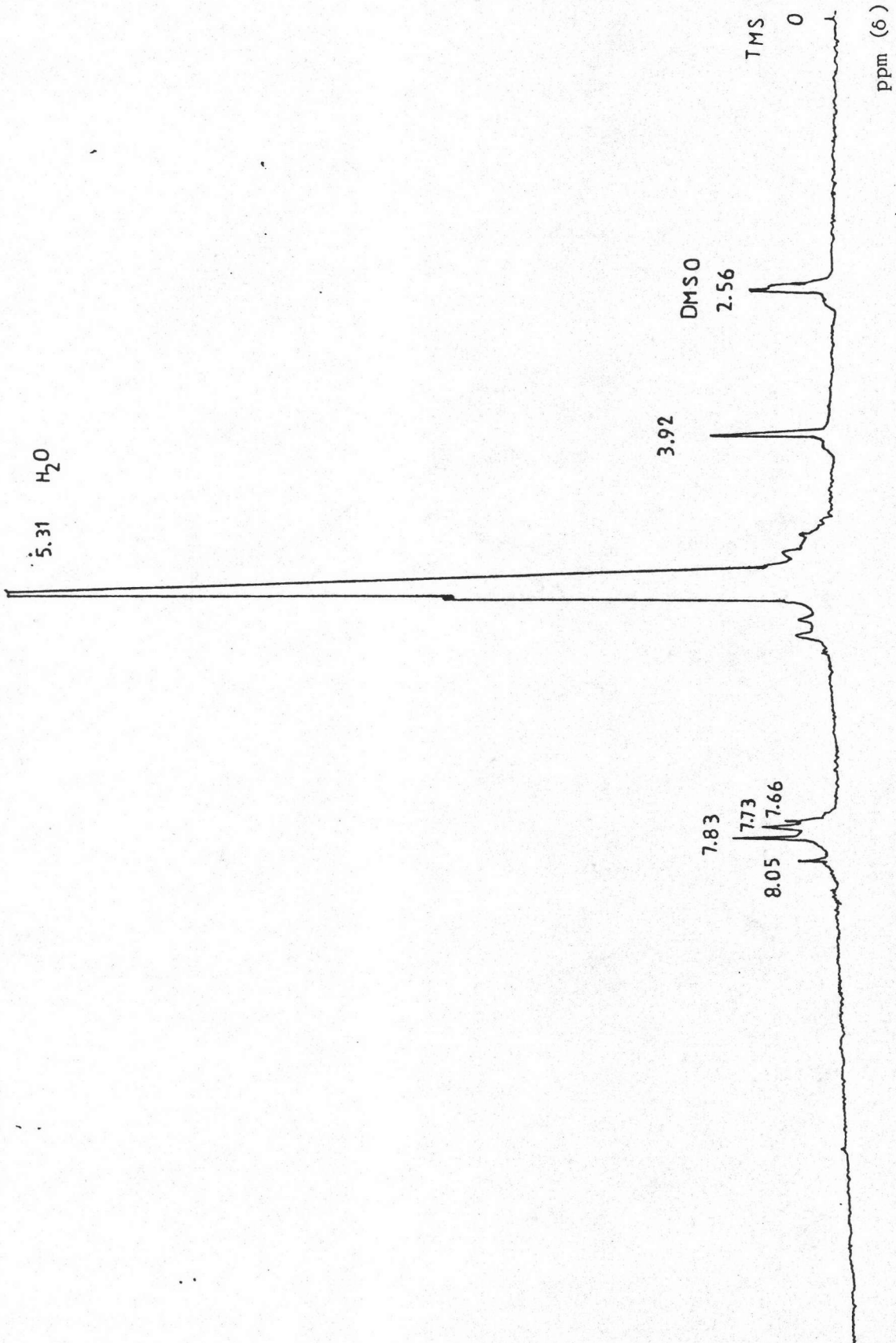


Figure 8 ¹H-nuclear magnetic resonance spectrum of mebendazole in acidified DMSO-d₆

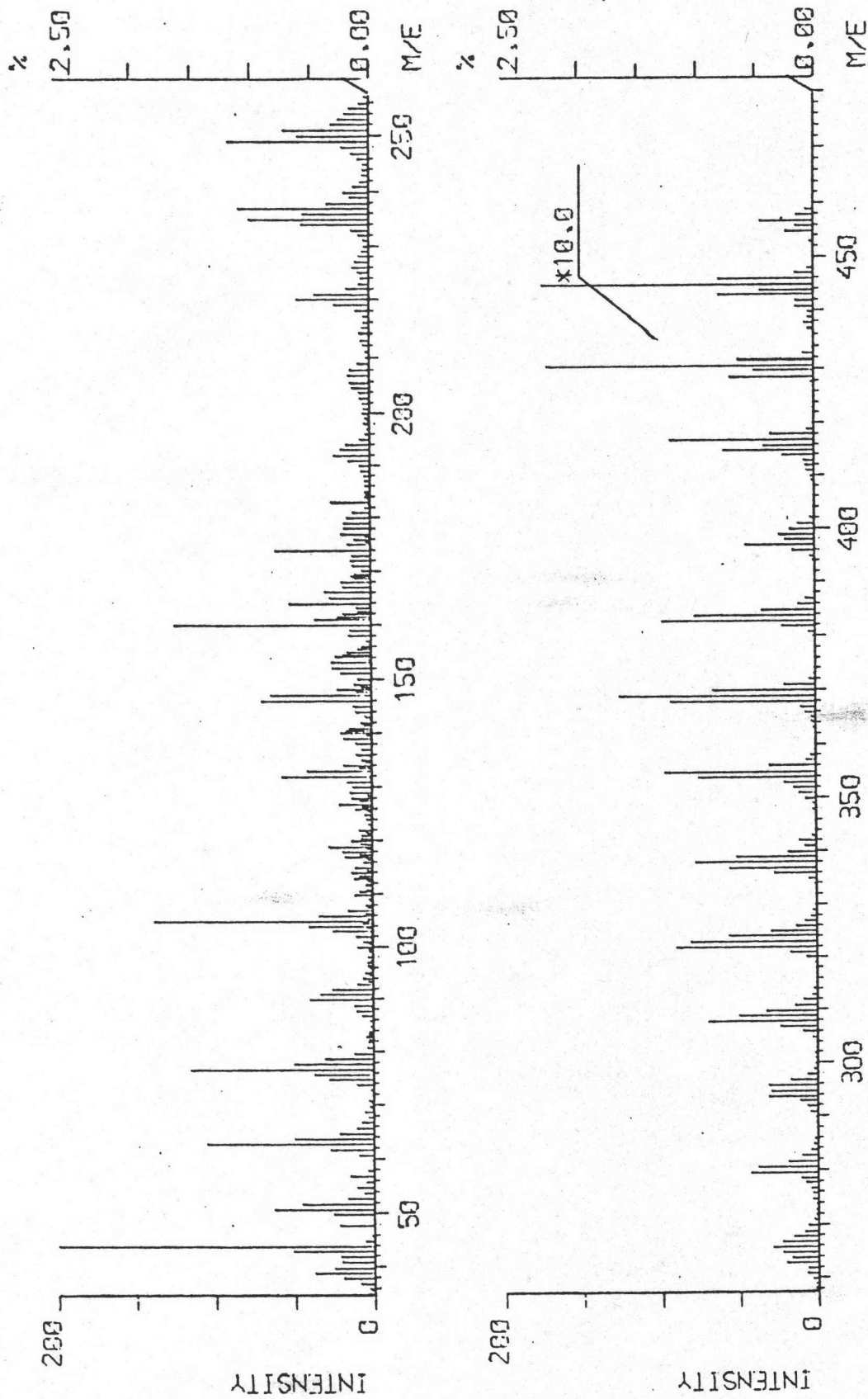


Figure 9 Mass spectrum (EIMS) of 2,4-dinitrophenylhydrazone derivative

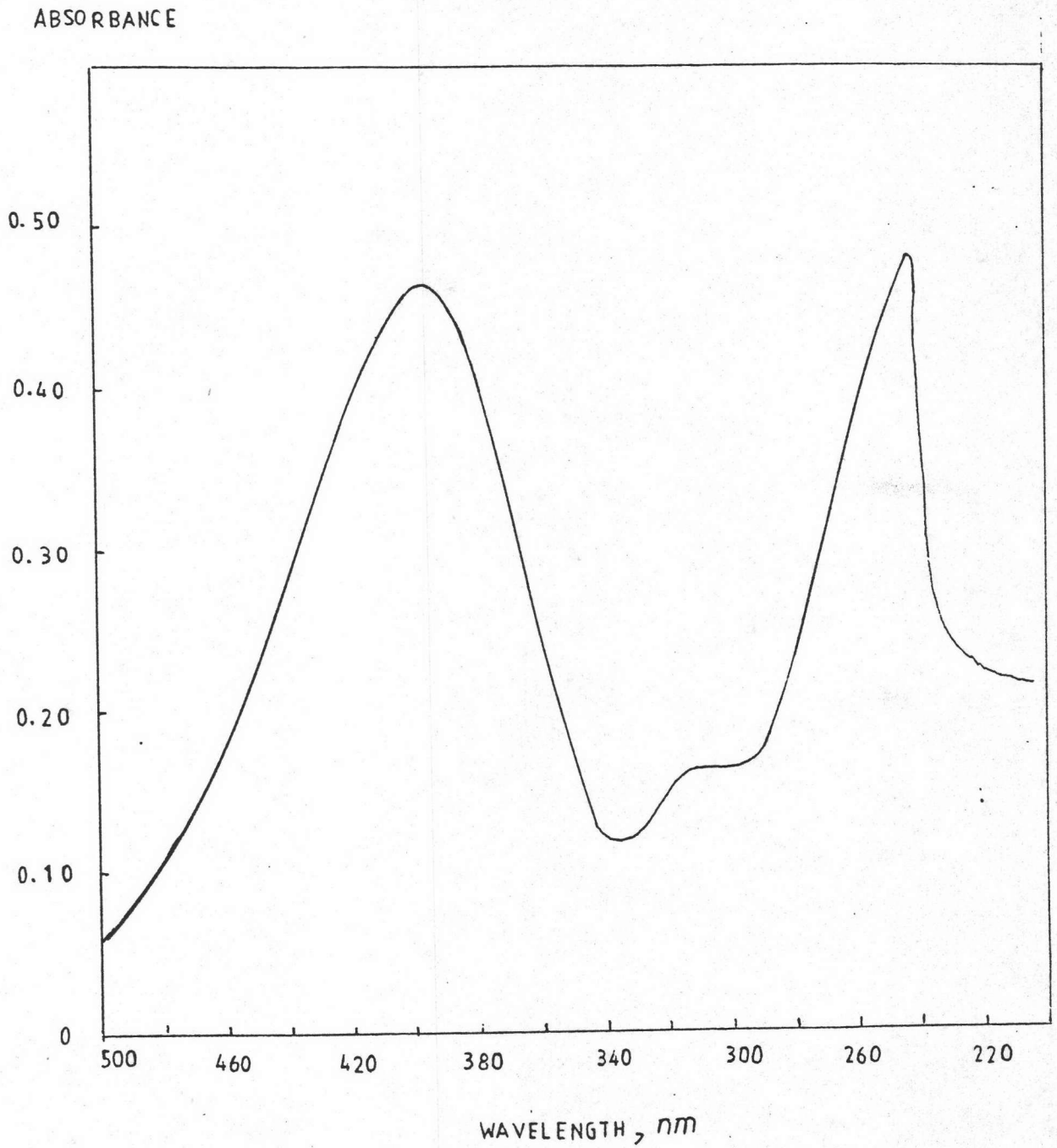


Figure 10 Absorption spectrum of 2,4-dinitrophenylhydrazone derivative in chloroform-methanol (2:1) mixture

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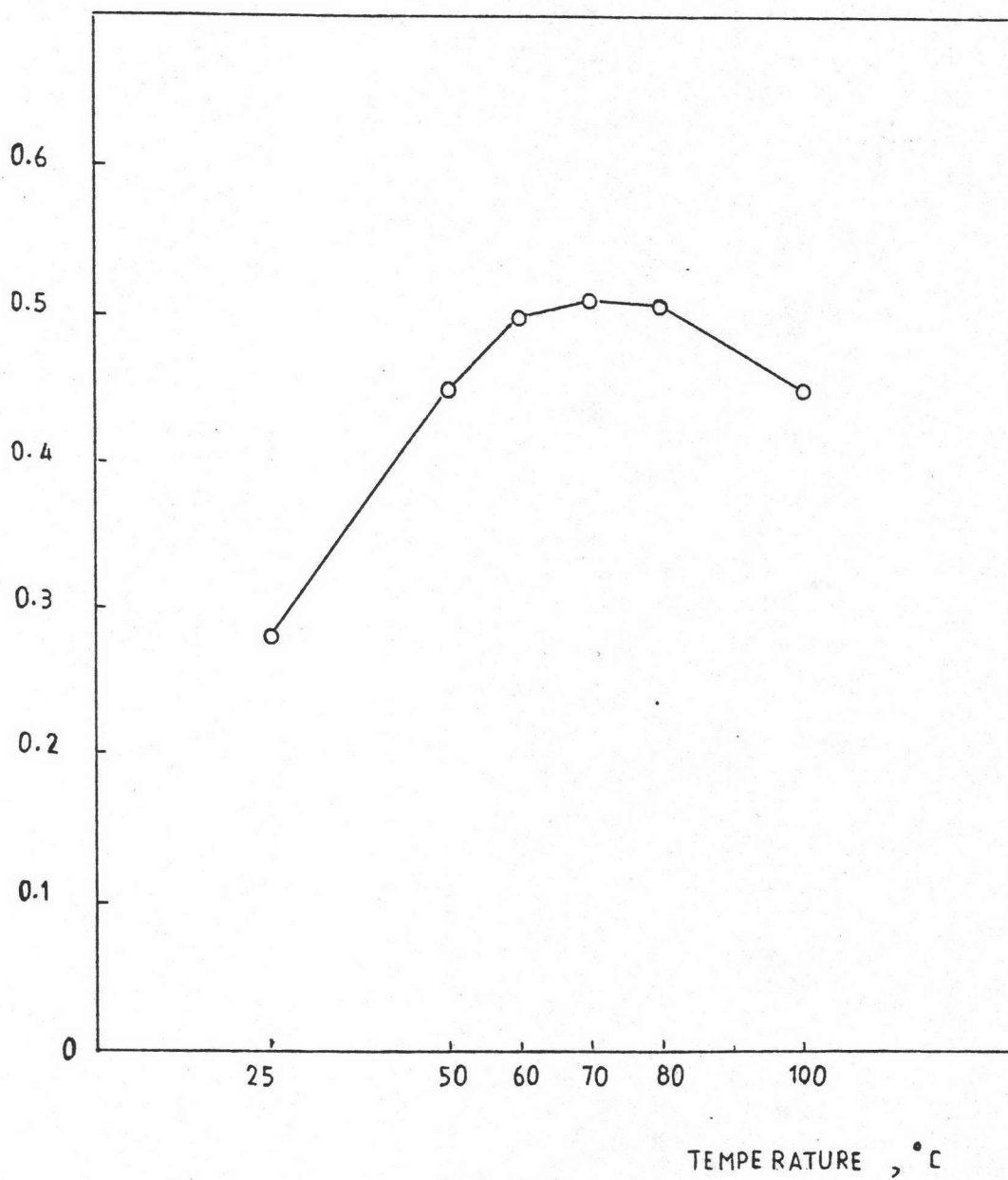


Figure 11 Effect of temperature on reaction of mebendazole and 2,4-dinitrophenylhydrazine

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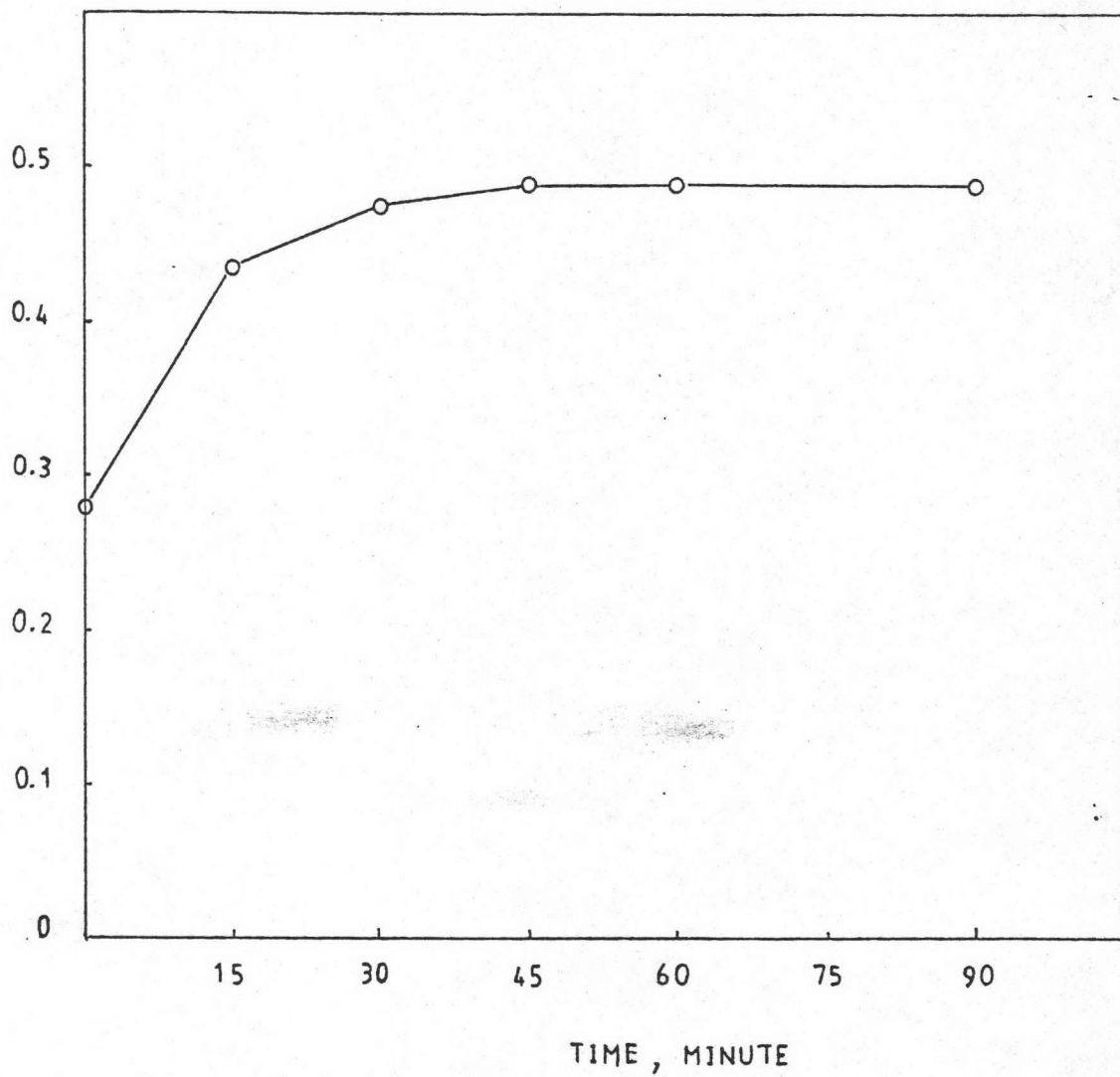


Figure 12 Effect of time on reaction of mebendazole and 2,4-dinitrophenylhydrazine at 70°C

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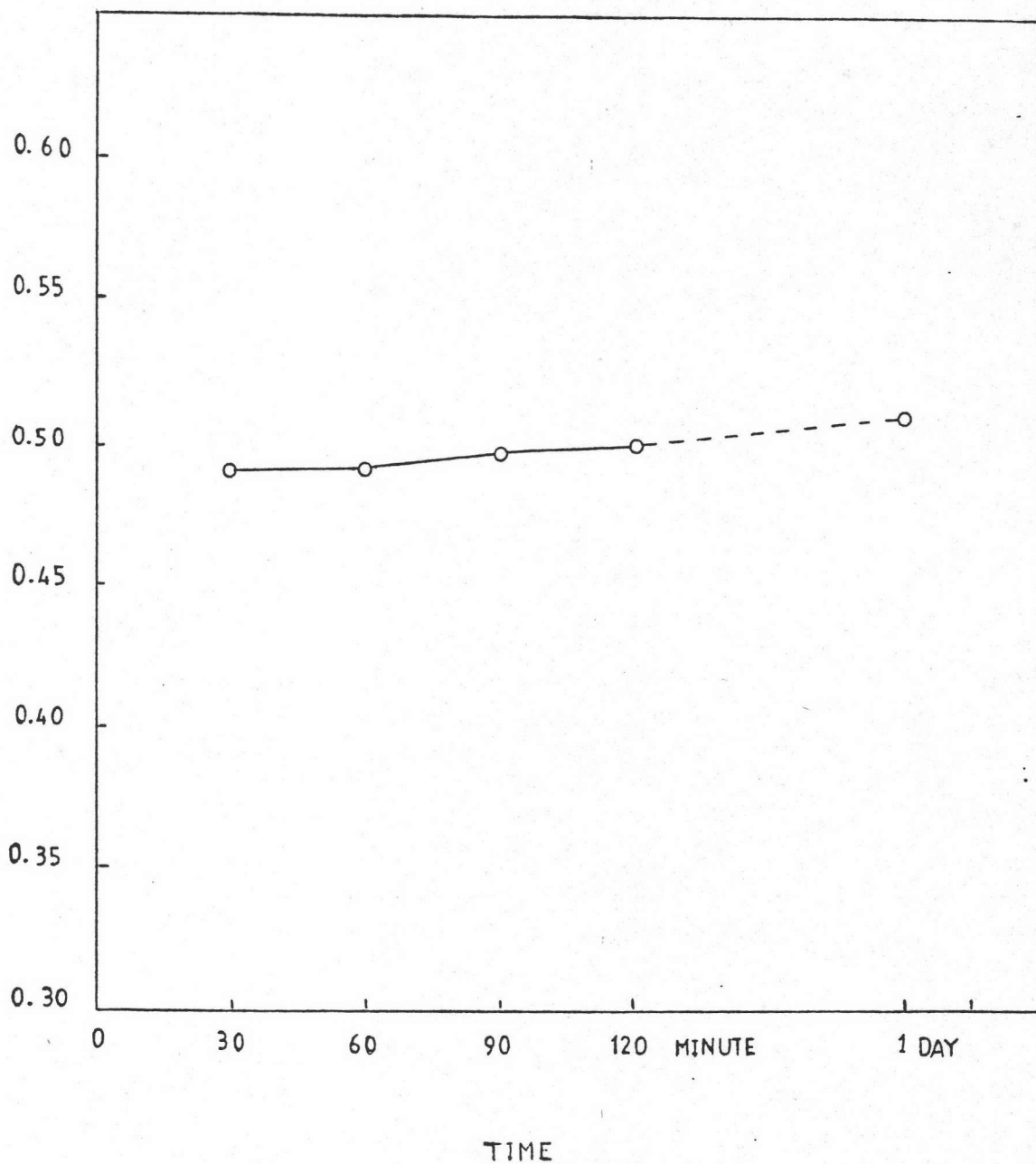


Figure 13 Effect of time for completed reaction of mebendazole and 2,4-dinitrophenylhydrazine at room temperature

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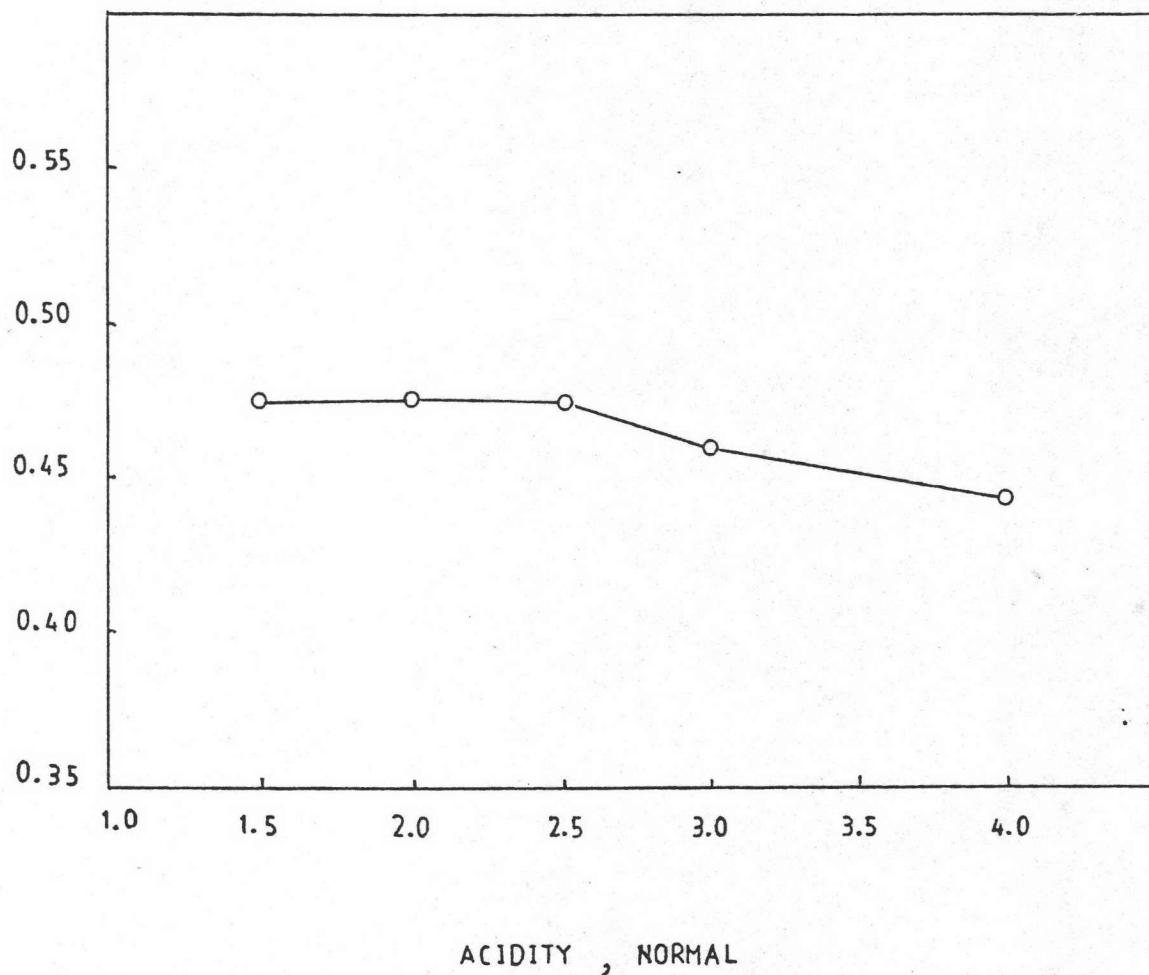


Figure 14 Effect of acidity on reaction of mebendazole and 2,4-dinitrophenylhydrazine

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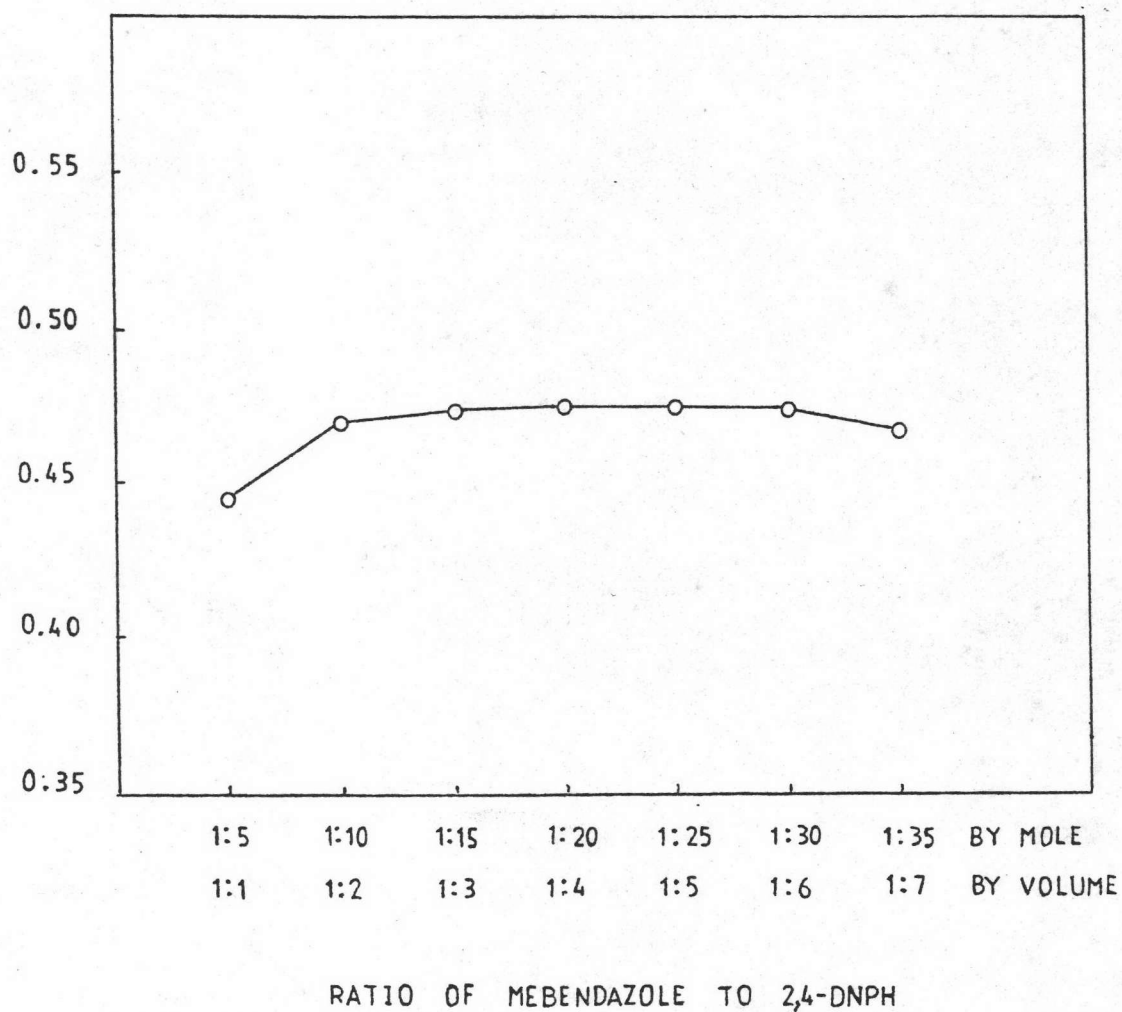


Figure 15 Ratio of mebendazole to 2,4-dinitrophenylhydrazine by mole and by volume (ml/ml)

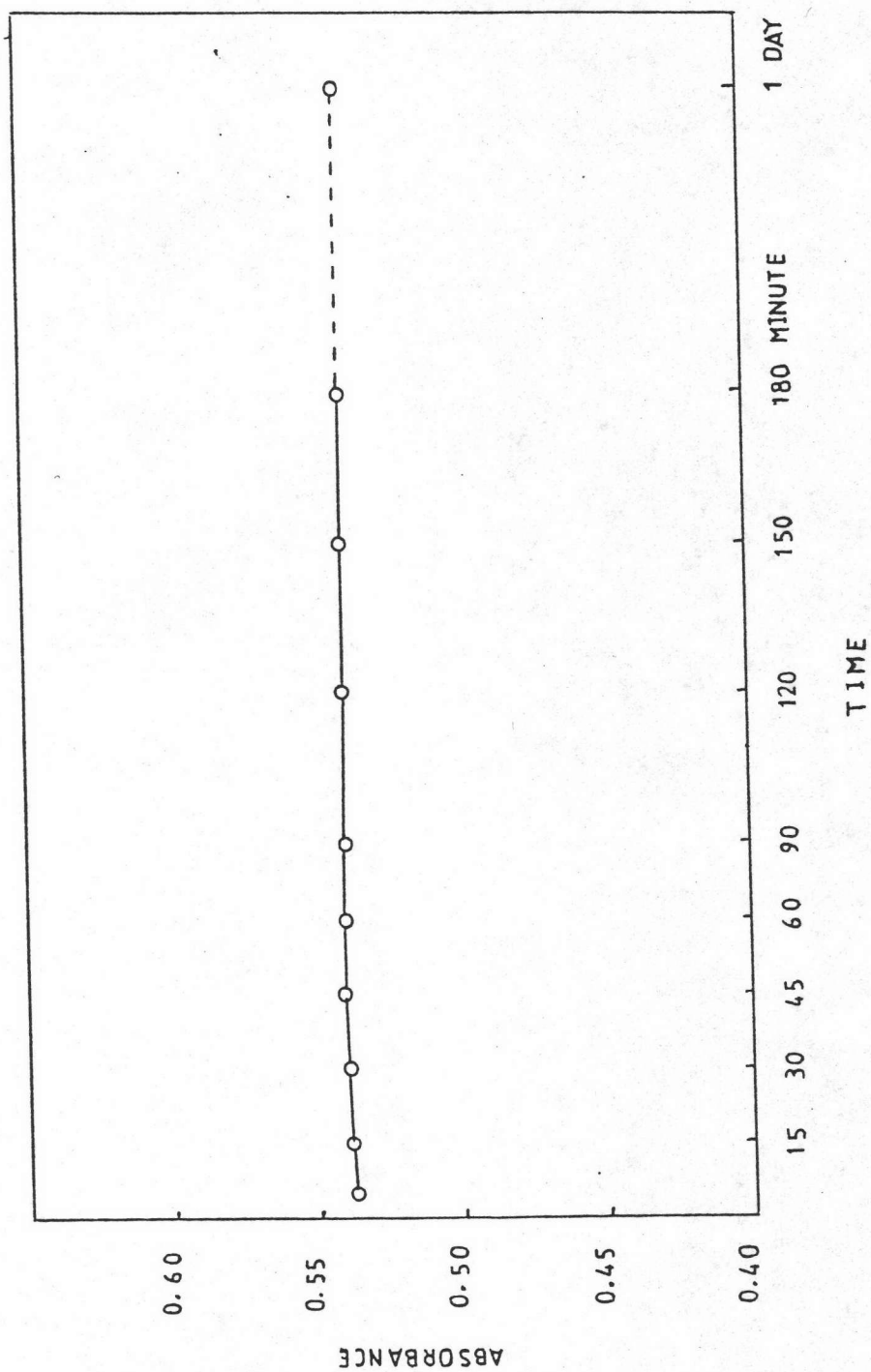


Figure 16 Effect of time on stability of 2,4-dinitrophenylhydrazone derivative

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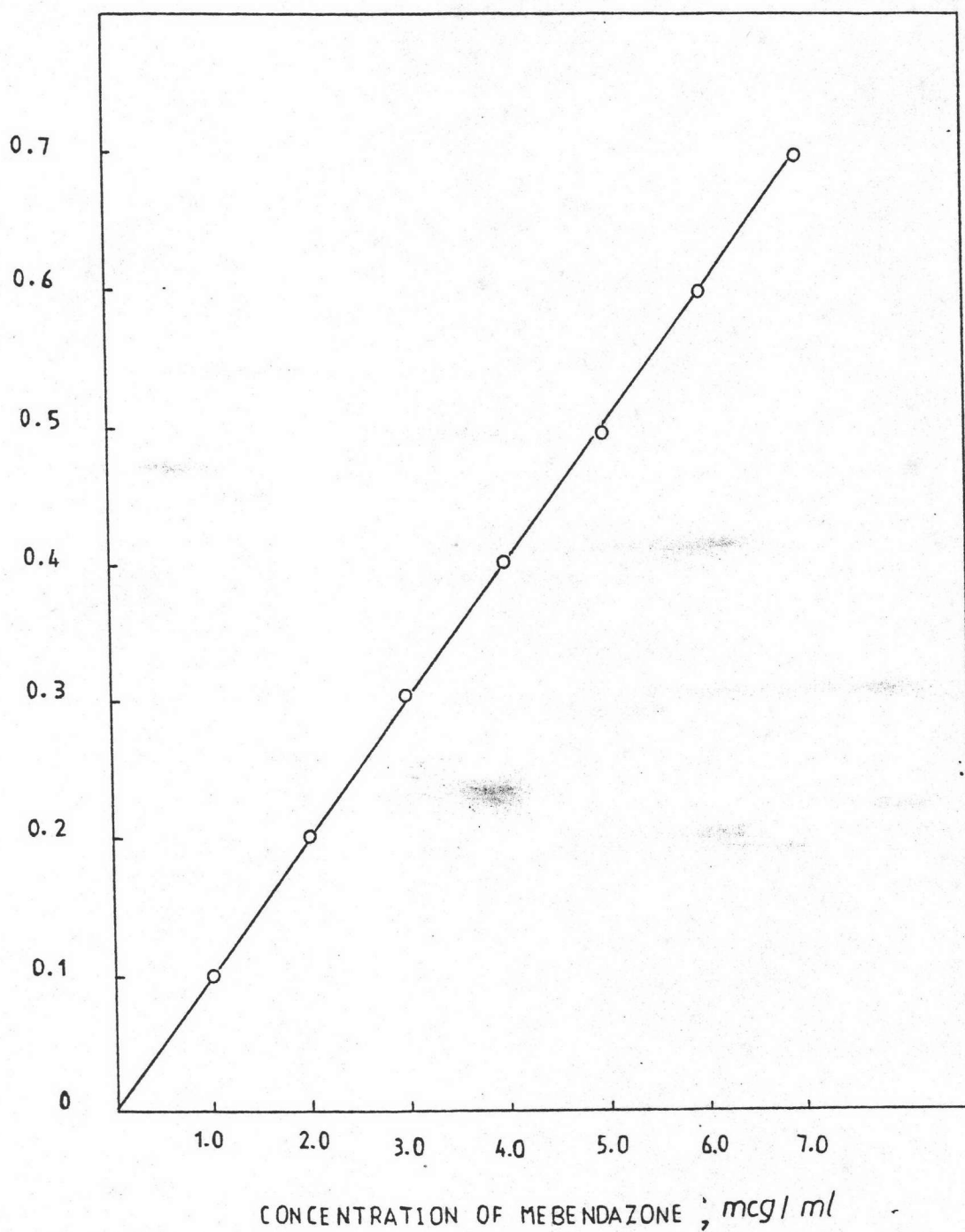


Figure 17 Linearity of Absorbance against concentration of mebendazole

VITA

Miss Siriporn Supakankunti graduated with a Bachelor of Science in Pharmacy in 1982, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

