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EFFECTS OF N-BEARING SALTS ON GC ANALYSIS OF MCPA AND ITS METABOLITES IN SOILS

M.A. SATTAR

Department of Soil Science, Bangladesh Agricultural University, Mymensingh, Bangladesh

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An internal standard GC technique was applied with MCPA (4-chloro-2- methylphenoxyacetic acid) and its two major metabolites 4-chloro-*o*-cresol and 5-chloro-3- methylcatechol by applying of PFB derivatization in presence of eight different nitrogen bearing salts. Except MCPA, the peak height gradually increased with increasing the levels of all eight salts. The peak areas and the recovery yields significantly increased with 4-chloro-o-cresol and 5-chloro-3-methylcatechol. The results were applied to the soils. Ammonium chloride produced the best yields. The detection limit was between 0.001 to 0.005 ppb.

Key words: MCPA, 4-chloro-*o*-cresol, 5-chloro-3 methylcatechol, N-bearing salts, Peak height, Peak areas, GC, PFB derivatization.

Introduction

MCPA (4-chloro-2-methylphenoxyacetic acid, I, Fig. 1) is a systemic foliar herbicide used against different species of weeds in the crop field and bush control in the forest [1]. The 4- chloro-*o*-cresol (II) and 5-chloro-3-methylcatechol (III) are among the two major metabolites of MCPA (I) by microorganisms [2- 4]. Zero [5] reported the GC method for MCPA and 2, 4-D in wheat and grain samples. Some methods described the GC of MCPA and/or other pesticides [6-8]. The TLC of MCPA and its metabolites has also been reported by some investigators [9-10]. Pentafluorobenzyl promide (PFB) derivatization has been largely applied with MCPA [11] and MCPA with its metabolites from the environmental samples [12-16], and these methods were largely applied to detect the metabolites from the environmental samples [17-20]. The metabolites of MCPA (II,III) are harmful to the human health and the environment, and such metabolites are coming to the environment because of the severe application of MCPA in agriculture and waste products of the paper industry. So a sensitive method is important for 4-chloro-o-cresol and 5-chloro- 3-methylcatechol, and the present study describes such a method.

Materials and Methods

Compounds. The following four compounds were used in the experiment.

I. MCPA (4-chloro-2-methylphenoxyacetic acid).

II. 4-Chloro-o-cresol.

III. 5-Chloro-3-methylcatechol.

IV. 2,6-Dimethoxphenyl (internal standard).

The compounds are 100% pure, and 0.1% solution of each was prepared with ether. The structure of the compounds (I-IV) and their PFB derivatization (V-VII) are reported in Fig. 1.

Nitrogen-bearing salts. Eight nitrogen bearing salts were tested in the PFB derivatization reaction of the compounds. They are (1) Ammonium nitrate, (2) ammonium chloride, (3) ammonium carbonate (4) sodium nitrate, (5) calcium nitrate, (6) ammonium sulphate, (7) mono-ammonium phosphate, (8) diammonium phosphate. 1% solution of each salt was prepared in distilled water.

Derivatization. Exactly 100,200 and 250 µl of I, II and III compounds were transferred, respectively, to a 15 ml glass-stoppered centrigfuge tube. Then 10 µl internal standard, 50, 100 and 500 µl of each N-salts, 30 µl 1% potassium carbonate and 1 ml of acetone were added and kept for reaction for one hour at room temperature (25 + 2°). The samples were evaporated by nitrogen stream. The samples were transferred to 10 ml volumetric flask with ether and ether added upto the mark.

Soil experiment. Five and 10 gm soil was treated with I, II and III compounds as above. They are mixed uniformly in presence of 1 or 2 ml of water. The samples were then extracted with Ether- acetone-hexane-heptane (2:1:1:1) by a

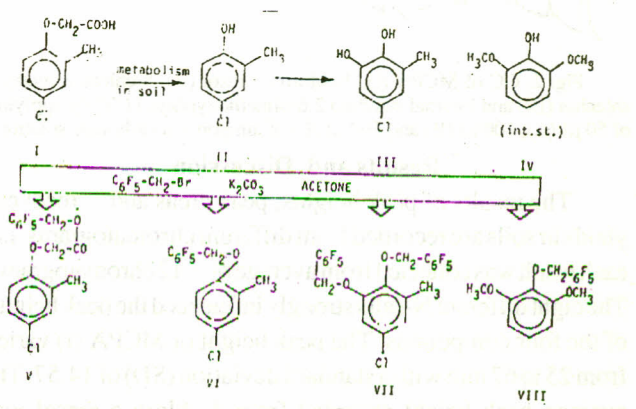

Fig. 1 Structure of MCPA (I), its metabolites 4-chloro-o-cresol (II) and 3-methyl-5-chlorocatechol (III), 2,6-dimethoxyphenyl (IV); internal standard of the analysis) and their pentafluorobenzyl derivatives (V-VIII).

shaking method as described by Sattar [21]. Then an aliquot of the soil extracted was transferred to a 15-ml glass stoppered centrifuge tube and applied for PFB derivatization as described above.

Water-toluene cleanup. An aliquot of the derivative sample was taken to a 15 ml glass-stoppered centrifuge tube. Then 5 ml distilled water and 10 ml toluene were added, mixed well, and the layer was separated and then injected to the GC after necessary dilution or redilutions.

GC determination. A varian Model 2400 gas chromatograph with 3H ECD was used. One or two µl of the toluene sample was injected with the split to a 25 m long glass capillary column and with external diameter 0.3 mm, coated with SE 30 silicone phase. Nitrogen was used as carrier gas 1 ml/min. Injector and detector temperatures were both 210°. A programmed temperature was applied from 120 to 200° with an increasing rate of 6°/min. Examples of GC chromatograms obtained are illustrated in Fig. 2.

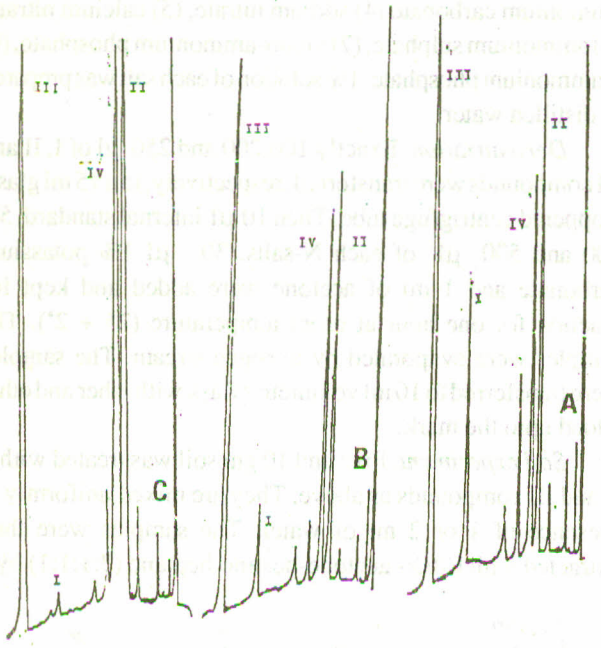

Fig. 2. GC of MCPA (I), 4-chloro-o-cresol (II), 5-chloro-3-methyl-catechol (III) and internal standard 2,6-dimethoxyphenyl (IV) by applying of 50 µl (A), 100 µl (B) and 500 µl (C) of ammonium carbonate solution.

Results and Discussion

The results of peak heights, peak areas and recovery yields in soils are recorded from different chromatograms, i.e. each result was collected from average of 3-12 chromatograms. The eight different N-salts strongly influenced the peak heights of the four compounds. The peak height of MCPA (I) varied from 25 to 67 mm with a standard deviation (SD) of 14.57. The average peak height recorded from 4-chloro-o-cresol was 113 mm with a variation of 71 to 140 mm (SD=23.69).5-chloro-3-methylcatechol (III) produced the peak heights from 122 to 70 mm (SD=17.45) and 96to 175 mm of the internal standard IV with SD value of 27.40 (Table 1). The average peak areas are recorded in Table 2. The results showed that the peak areas largely increased with the increasing levels of N-salts in II, III and IV compounds, but the peak areas sharply decreased with the increasing levels of all (1 - 8) N-salts. This is because of the presence of large amount of water in the salts

TABLE 1.AVERAGE PEAK HEIGHTS (mm) OF MCPA (I) AND ITS METABOLITES 4-CHLORO-*o*-CRESOL (II) 5-CHLORO-3 METHYLCATECHOL (III) AND THE INTERNAL STANDARD 2,6-DIMETHOXYPHENYL (IV) WITH DIFFERENT N-BEARING SALTS (EACH AVERAGE OF 12 CHROMATOGRAMS).

N-Salts tested	Compounds			
	I	II	III	IV
1. Ammonium nitrate	32	71	132	96
2. Ammonium chloride	63	136	157	128
3. Ammonium carbonate	25	83	137	104
4. Sodium nitrate	53	113	167	123
5. Calcium nitrate	37	140	138	131
6. Ammonium sulphate	35	135	170	172
7. Monoammonium phosphate	67	107	167	113
8. Diammonium phosphate	55	120	122	123

TABLE 2. THE AVERAGE PEAK AREAS OF MCPA(I), 4-CHLORO-o-CRESOL (II) 5-CHLORO-3-METHYLCATECHOL (III) AND THE INTERNAL STANDARD 2,6-DIMETHOXY-PHENYL (IV) BY APPLYING OF DIFFERENT CONCENTRATIONS OF N-BEARING SALTS

N-Salts tested	Amount applied µl/sample	Compounds			
		I	II	III	IV
1. Ammonium nitrate	50	70	120	70	60
	100	68	130	135	68
	500	10	200	195	110
2. Ammonium chloride	50	100	170	252	110
	100	80	190	277	130
	500	15	200	279	175
3. Ammonium carbonate	50	50	195	120	55
	100	15	210	150	65
	500	5	255	195	130
4. Sodium nitrate	50	142	120	155	88
	100	50	170	175	115
	500	15	180	215	187
5. Calcium nitrate	50	85	165	170	112
	100	22	248	210	140
	500	8	267	252	180
6. Ammonium sulphate	50	70	160	270	100
	100	30	180	278	140
	500	10	270	285	165
7. Monoammonium phosphate	50	180	240	90	90
	100	70	255	135	98
	500	10	270	190	135
8. Diammonium phosphate	50	100	210	110	130
	100	50	240	128	135
	500	10	245	170	145

which is strongly affected by the ECD in GC. Monoamonium is highly sensitive to MCPA, where the peak areas decreased from 180 to 10 by applying of 50 and 500 µl of the solutions in the PFB derivatization. Again the same monoammonium salt (7) produced the maximum peak area yields with 4-chloro- ocresol with all the three levels applied (240-270), and the average being recorded to 255 and the lowest was measured with ammonium nitrate (150). In case of 5-chloro-3-methylcatechol (III) the average peak areas measured from 136 (No. 8) to 277 (No. 4). The sensitivity of peaks are greatly increased with increasing the concentrations of the salts. The internal standard 2,6-dimethoxyphenyl (IV) also produced sharp influence with all N-salts where the average peak areas observed from 79.33 (No. 1) to 144 (No. 7). (These N-salts seem to have catalytic effect which disturbs the formation of PFB derivatization of a carboxylic acid (such as MCPA) but does not prevent, may be even aaids derivatization of the phenolic group (in compounds II-IV). The peak areas of MCPA(I) and its metabolites (II, III) relative to internal standard (IV) are collected in Table 3. The variations were recorded from 0.27 (No. 4,6) to 0.81 (No. 7) for MCPA (SD=0.06) 1.14 (No. 5) to 2.64 (No. 2) for 4-chloro-o-cresol (S.D=0.48) and 0.99 (No. 8) to 2.06 (No. 4) for 5-chloro-3-methylcatechol (SD=0.34). Table 4 shows the average percent recovery yie lds of I, II and III with model compounds and soil samples. The results showed that MCPA (I) gave the poor recovery yields (30-5%) both in model compounds and soil samples. The fact is already explained. The presence of water with N-salts first influence the derivatization and then to ECD ofGC particularly with phenoxy herbicides (22). So this method is not good for single application of the MCPA or other phenoxyherbicides where a few excellent methods have already been reviewd. Usually the metabolite yields decreased gradually with time. So this procedure is very good to study or to detect the metabolites (II, III) from the environmental samples by creating the sensitivity of the

TABLE 3. PEAK AREAS MEASURED FOR MCP A (I) 4-CHLORO-O- CRESOL (II) AND 5-CHLORO-3-METHYLCATECHOL(III) RELATIVE TO INTERNAL STANDARD(IV)

N-Salts tested	Compounds		
	I	II	III
1. Ammonium nitrate	0.62	1.89	1.69
2. Ammonium chloride	0.28	2.64	1.86
3. Ammonium carbonate	0.47	1.33	1.94
4. Sodium nitrate	0.24	1.51	2.06
5. Calcium nitrate	0.50	1.14	1.38
6. Ammonium sulphate	0.27	1.57	1.45
7. Monoammonium phosphate	0.81	2.37	1.28
8. Diammonium phosphate	0.39	1.69	0.99

TABLE 4. THE AVERAGE PERCENT RECOVERY YIELDS OF MCPS (I), 4-CHLORO-O-CRESOL (II) AND 5-CHLORO-3-METHYLCATECHOL (III) FROM MODEL COMPOUNDS AND SOIL SAMPLES BY APPLYING OF HIGHEST CONCENTRATION (500 ml) OF THE SALT SOLUTION IN THE DERIVATIZATION, (EACH AVERAGE OF THERE CHROMATOGRAMS).

N-Salts applied	Recovery from model compounds			Recovery from soil samples		
	I	II	III	I	II	III
1. Ammonium nitrate	45	101	101	48	101	101
2. Ammonium chloride	30	102	102	33	102	102
3. Ammonium carbonate	35	100	100	36	100	100
4. Sodium nitrate	30	90	101	32	91	101
5. Calcium nitrate	48	75	85	50	89	89
6. Ammonium sulphate	30	99	80	32	99	81
7. Monoammonium phosphate	50	102	98	51	102	99
8. Diammonium phosphate	42	99	80	45	100	85

method. Most salts produced around 100% yields with II and III model compounds and soil samples. Here for group analysis or detection of metabolites of MCPA two N-salts ammonium nitrate (No. 1) and ammonium chloride (No. 2) are found to be best where the recovery yields were 101-102% in II and III compounds. For single study of either II or III several salts can be applied for better yields. The conclusion is that any N-salt sharply increased the sensitivity of II and III in soils. The detection limit was recorded from 0.001 to 0.005 ppb from the environmental samples.

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