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Gas chromatographic determination of triclopyr in fruits and vegetables

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Abstract

This research was comprised of two parts: quantitative analyses, and confirmatory test. In the quantitative analyses, five classes of fruits and vegetables comprising 10 individual commodities were fortified with triclopyr herbicide at 0.4 and 0.8 ppm level. Triclopyr was extracted from the matrices and derivatized separately to 2-chloroethylene ester with 2-chloroethanol–BCl_3 and methyl ester with diazomethane. The esters were then quantitated by GC–ECD and GC–NPD. The GC–ECD recoveries for 2-chloroethylene ester were 100.0% and 100.7% at 0.4 ppm and 0.8 ppm fortification levels, respectively, whereas methyl ester recovery was 103.9% at 0.4 ppm fortification level. Similarly, the GC–NPD recoveries for 2-chloroethylene ester were 99.0% and 97.9% at 0.4 ppm and 0.8 ppm fortification levels respectively, whereas methyl ester recovery was 102.0% at 0.4 ppm fortification level.

In the confirmatory test, the 2-chloroethylene ester was introduced into a GC–ion trap. The EI mass spectrum was then interpreted based on the criteria of molecular ion, isotopes, base ion, characteristic ions and the nitrogen rule. Compared to existing methods, this method has reduced partition solvents to nearly one-tenth. In addition, this method proved to be simple, fast, safe and accurate.

1. Introduction

Triclopyr is a systemic herbicide manufactured by DowElanco, its chemical name is 3,5,6-trichloro-2-pyridyloxyacetic acid by IUPAC [1]. It is often used in mixed formulation with other phenoxyalkanoic acids (e.g. 2,4-D, dicamba, mecoprop, etc.) for control of woody plants and many broad leaved weeds in grasslands, uncultivated lands, and rice fields [1,2]. As the rice market becomes more liberal in Japan and other Asian countries, California rice could become an important export in the near future. Consequently, rice production related chemicals, such as triclopyr, may be used more frequently than in the past. As a result, the occurrence of illegal applications and/or other crop violations due to drift from adjacent rice fields would rise. Therefore, the development of an effective and accurate analysis method is crucial for a regulatory agency like ours.

Conventionally, triclopyr is analyzed like phenoxyalkanoic herbicides [3–5]. This method requires a large amount of solvents for partition clean-up in sample extraction. Additionally, diazomethane, which is both explosive and exceed-

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ingly toxic [6], must be generated on site in the existing method for the methyl ester derivatization reaction. Methyl ester is an early eluting compound which is vulnerable to certain commodity (e.g. citrus, onion and cabbage) matrix interferences. Chromatographically, early eluting matrix interferences from fruits and vegetables create difficulty in identification and quantitation.

Therefore, the first objective in method development was to reduce the volume of solvents in the sample preparation procedures. The second objective was to replace diazomethane with a derivatizing reagent that was (1) comparatively non-explosive, (2) low in toxicity, (3) an enhancement for detector response, (4) an improvement for separation and/or resolution (longer retention time or late peak) and (5) equivalently or better interpreted by an advanced instrument such as a mass spectrometer. The third objective was to establish an accurate method which was confirmed by applying multiple instrumentation strategy. In the method, we use GC–ECD and GC–NPD for quantitation and GC–ion trap for confirmation. The multiple instrumentation approach would seem to be redundant; however, in case of enforcement action or litigation by a governmental agency such as ours, decisive scientific evidence beyond a shadow of doubt is necessary.

2. Experimental

2.1. Chemicals

The solvents used in the experiment were analysis-grade reagents from J.T. Baker. The sodium sulfate (Na_2SO_4) anhydrous granular was purchased from EM Science. The derivatization reagent, 2-chloroethanol–boron trichloride (10%), was purchased from Applied Science Laboratories. The triclopyr standard (100%) was supplied and purity certified by the California Department of Food and Agriculture (CDFA), standards repository.

2.2. Instrumentation

Three instruments were used in this study.

(a) A Varian 3300 GC system was equipped with a Ni63 electron capture detector (ECD). The temperature program was: initial temperature 150°C held for 2 min, ramp rate 10°C/min, 1st final temperature 180°C held for 7 min, ramp rate 20°C/min, 2nd final temperature 250°C held for 2 min. The injection mode was splitless, held for 0.75 min and the injection volume was 1 μl. A J&W Scientific DB-210 column (50% trifluoropropyl methyl poly siloxane) was installed for the study. The column size was 15 m × 0.32 mm I.D. and coated film thickness was 1.0 μm.

(b) A Perkin-Elmer Sigma 2 GC system was equipped with a thermionic nitrogen–phosphorus detector (NPD). The temperature program was: initial temperature 150°C held for 2 min, ramp rate 10°C/min, final temperature 225°C held for 2 min. The injection volume was 2 μl. A Hewlett Packard HP-1 column (methyl silicone gum) was installed. The column size was 10 m × 0.53 mm I.D. and coated film thickness was 2.65 μm.

(c) A Varian 3400 GC system was equipped with a Finnigan MAT ITS-40 mass spectrometry detector. The temperature program was: initial temperature 150°C held for 1 min, ramp rate 10°C/min, final temperature 280°C held for 6 min. The injection mode was splitless, held for 0.75 min and the injection volume was 2 μl. For the ion trap ITS-40 system, the parameters were: mass range 50 to 400 amu, 1 second/scan, acquire time 20 min, fil/mul delay 240 s, peak threshold 50 counts, mass defect 50 mmu/100 amu, background mass 49 amu, ionize mode EI, and auto ion control on. A J&W Scientific DB-5 column (5% phenyl methyl poly siloxane) was used. The column size was 30 m × 0.25 mm I.D. and coated film thickness was 0.25 μm.

2.3. Preparation of spiked produce samples

Produce was grouped into 5 classes consisting of leafy vegetables, roots, fruits, citrus and spices. Each group contained 2 commodities in

the experiment. A unit of 3 Mason jars with 50 g per jar of chopped commodity was prepared for fortification. The first jar was fortified with 20 μg triclopyr (0.4 ppm level) for diazomethane derivatization as the control. The second and third jars were spiked with 20 μg and 40 μg triclopyr, respectively, to form 0.4 ppm and 0.8 ppm level for 2-chloroethanol–BCl_3 ethylation studies.

2.4. Sample extraction, derivatization and micro partition clean-up procedure

Vegetable or fruit was chopped and mixed in a food chopper. Only 50 g of the well chopped sample was placed in a 1-pt (470-ml) Mason jar. Then, 10 ml H_2SO_4 (1:1 in H_2O), 50 ml ethyl ether–hexane (1:1) and 25 g Na_2SO_4 were added into the jar. The contents of the jar were then blended by an Omni-Mixer for 2 min. The entire mixture was transferred into a 200-ml wide-mouth centrifuge bottle and the bottle was centrifuged for 2 min at 201 *g* (1500 rpm). The top 5 ml ethyl ether–hexane layer was pipetted into a 15-ml conical tube and evaporated to ca 0.5 ml with gentle air concentrator (N- EVAP) at ambient temperature.

Methylation

Approximately 0.25 ml or 10 drops of diazomethane was added into the conical tube. The tube was capped, and the contents were mixed and allowed to react for 30 min.

Ethylation

Approximately 0.2 ml or 7 drops of 2-chloroethanol–BCl_3 was added into the conical tube. The tube was capped, and then the cap was wrapped with a piece of parafilm so that it was sealed. The contents were mixed and allowed to react in a water bath at 70°C for 30 min. A volume of 5 ml of hexane was transferred into the conical tube and thoroughly mixed to dissolve methyl or ethylene ester.

Micro partition clean-up procedure

Approximately 5 ml of distilled water was added into the conical tube, mixed and allowed to settle for a couple of minutes. The bottom aqueous layer was discarded using a disposable pipette. This partition clean-up step was repeated two more times. Approximately 1.5 g of Na_2SO_4 was added into the tube to prepare it for GC injection. For GC–ion trap analysis, 3 ml of the solute was concentrated to 0.3 ml before injection.

3. Results

3.1. The quantitative analyses by GC–ECD and GC–NPD

The herbicide residues 0.4 ppm and 0.8 ppm were fortified into five classes of fruits and vegetables based on experimental design by Ting and Kho [7]. The 2-chloroethylene esters were then tested by GC–ECD and GC–NPD methods and the recovery data were compared with diazomethane derivatized methyl ester results in Tables 1 and 2.

In Table 1, the recovery means were 100.0% (0.4 ppm) and 100.7% (0.8 ppm) for 2-chloroethylene esters and 103.9% (0.4 ppm) for methyl esters. All of the results were excellent; however, in the comparison of R.S.D.s (13.5% and 3.5% for 2-chloroethylene esters versus 17.3% for methyl esters) there was a slight advantage when using 2-chloroethylene esters. This was because 2-chloroethylene esters had later eluting peaks; there was longer retention time, better separation, better resolution and less interference from matrices. This is illustrated by the orange samples in Figs. 1 and 2. The methyl ester had a retention time of 3.65 min which almost overlapped the co-eluting interference peak at 3.84 min in Fig. 1. Conversely, in Fig. 2, the 2-chloroethylene ester had a longer retention time, $t_R = 7.35$ min, and was totally separated from early eluting matrix interferences.

Triclopyr is a herbicide containing a pyridine ring; in addition to GC–ECD, GC–NPD is also a logical tool to be investigated. The recovery data are presented in Table 2. Again, the advantage of using 2-chloroethylene ester is demon-

Table 1
Recovery data (%) by GC–ECD analyses

Vegetables and fruits		2-Chloroethylene ester		Methyl ester
		0.4 ppm	0.8 ppm	0.4 ppm
Leafy vegetables	Lettuce	90.2	99.5	101.1
	Cabbage	97.2	103.6	96.4
Roots	Carrot	101.3	94.5	95.4
	Potato	105.9	99.8	98.7
Fruits	Apple	81.6	102.5	89.9
	Cantaloupe	102.8	105.6	99.1
Citrus	Orange	97.1	104.3	98.6
	Lemon	96.6	100.6	99.9
Spices	Green Onion	133.1	100.6	153.6[a]
	Pepper	94.5	96.2	105.9
Statistics	Mean	100.0	100.7	103.9
	S.D. (n = 10)	13.5	3.5	17.9
	R.S.D.	13.5	3.5	17.3

[a] Elevated result due to coeluting interferences from matrix.

Table 2
Recovery data (%) by GC–NPD analyses

Vegetables and fruits		2-Chloroethylene ester		Methyl ester
		0.4 ppm	0.8 ppm	0.4 ppm
Leafy vegetables	Lettuce	100.1	95.9	105.9
	Cabbage	96.7	88.9	123.2[a]
Roots	Carrot	101.4	99.5	97.7
	Potato	90.3	107.9	101.6
Fruits	Apple	101.6	95.2	99.7
	Cantaloupe	104.0	88.2	104.1
Citrus	Orange	91.0	106.5	95.7
	Lemon	94.1	92.0	104.9
Spices	Green Onion	102.0	102.0	97.9
	Pepper	108.6	102.9	89.3
Statistics	Mean	99.0	97.9	102.0
	S.D. (n = 10)	5.9	7.0	8.9
	R.S.D.	5.9	7.1	8.8

[a] Elevated result due to coeluting interference from matrix.

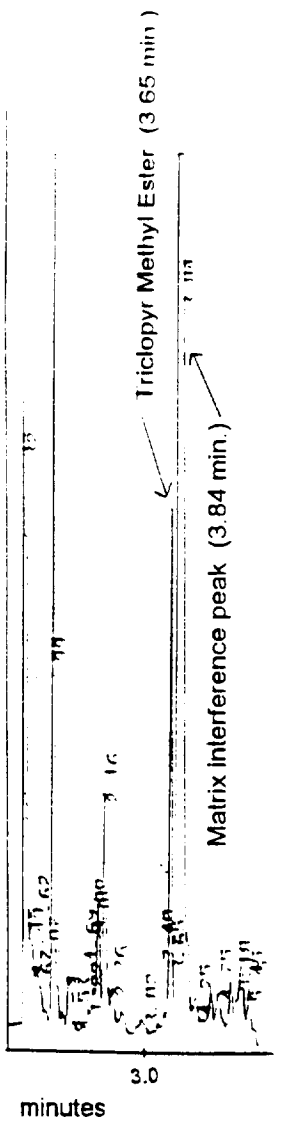

Fig. 1. Chromatogram of triclopyr methyl ester in orange by GC–ECD.

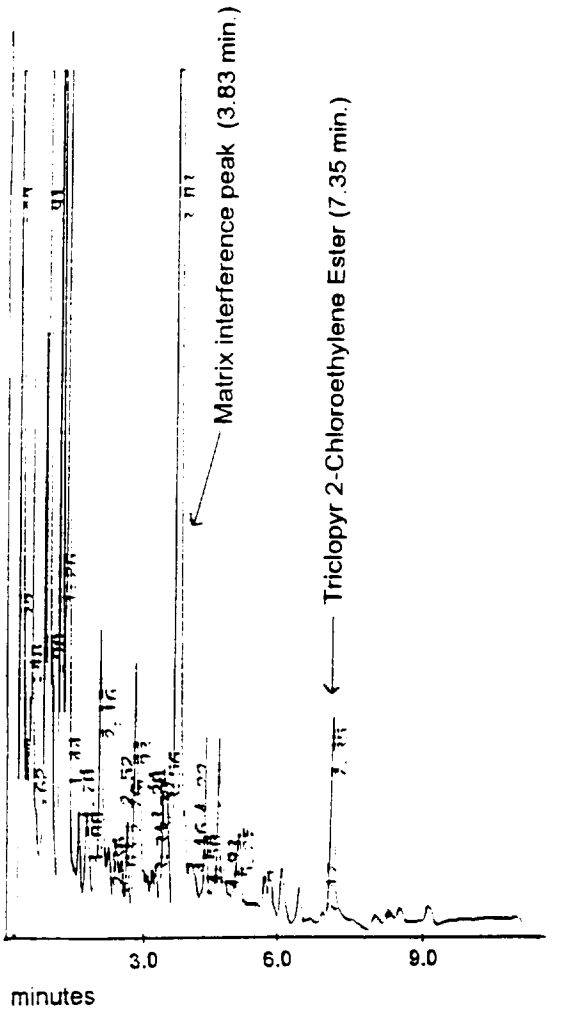

Fig. 2. Chromatogram of triclopyr 2-chloroethylene ester in orange by GC–ECD.

strated in the nitrogen rich commodity cabbages. The recovery for 2-chloroethylene ester in cabbage was 96.7%, which was better than the 123.2% for methyl ester at the same fortification level, 0.4 ppm. The elevated recovery result occurred as the methyl ester peak at 3.556 min had a shorter retention time (Fig. 3); it was thus susceptible to co-eluting interferences in the matrix. Meanwhile, the 2-chloroethylene ester peak at 6.729 min in Fig. 4 was completely separated and had better resolution because of the longer retention time. Therefore, the recovery of 2-chloroethylene ester was much more reasonable. Overall, the GC–NPD data (99.0% and 97.9% for 2-chloroethylene esters versus 102.0% for methyl ester) were as good as the GC–ECD as regards the recovery mean percentages. Keep in mind that the GC–NPD was not affected by chloride interferences from derivatizing reagents and consequently, the background noise was far less for the GC–NPD in comparison to the GC–ECD. Therefore, at the

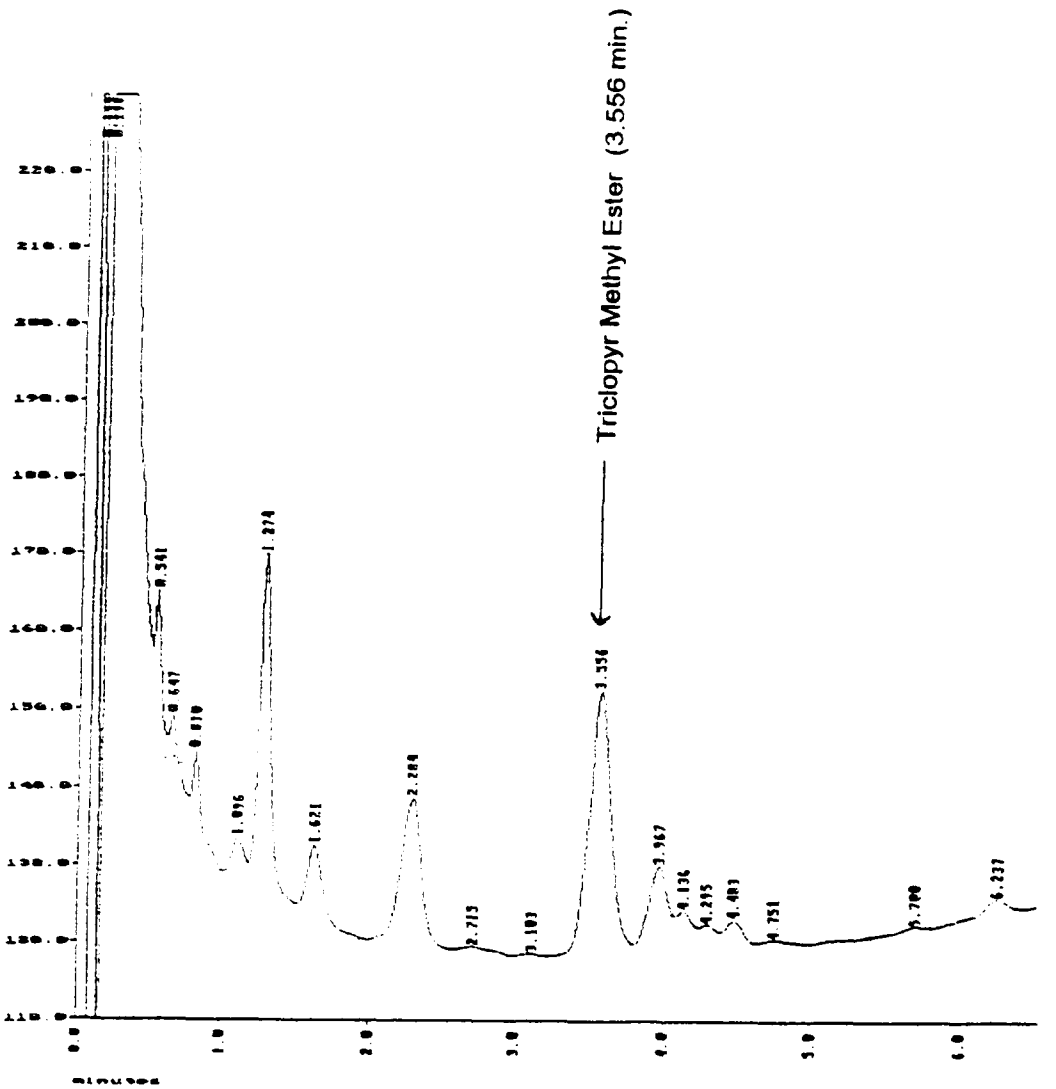

Fig. 3. Chromatogram of triclopyr methyl ester in cabbage by GC–NPD.

lower fortification level (0.4 ppm), the R.S.D. data for GC–NPD appeared less variable, e.g. $8.8 < 17.3$ and $5.9 < 13.5$.

One point worth mentioning is that the signal-to-noise ratio in the chromatograms was generally lower for GC–NPD than in GC–ECD analyses. This observation was to be expected since there is only one nitrogen atom on the pyridine ring in comparison to three or four chloride atoms, on methyl or ethylene esters, respectively. Therefore, the detection responses were restricted. If the fortified level had to be lower than 0.4 ppm, the GC–NPD method would consequently be more difficult to use.

Throughout the quantitative study, data were gathered from peak height rather then peak area. At low level residue analyses, the peaks were small, unsymmetrical and often distorted by background noise and/or matrix interferences. The impact was disastrous when peak

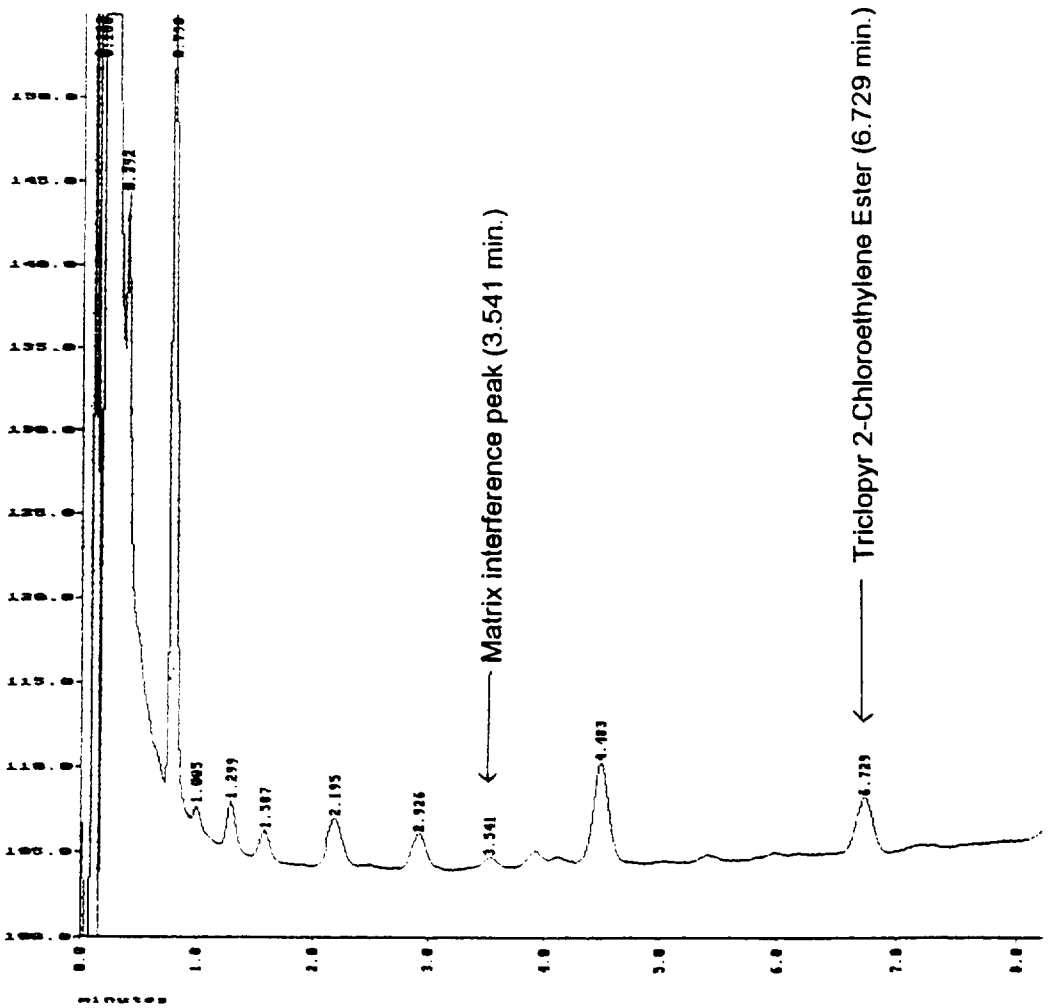

Fig. 4. Chromatogram of triclopyr 2-chloroethylene ester in cabbage by GC–NPD.

area was tried in the preliminary trials. Peak height was definitely the better data to take into account.

3.2. The qualitative or confirmation study by GC–ion trap

Triclopyr was first derivatized by 2-chloroethanol–BCl_3 and the ethylene ester synthesis was based on the acid chloride conversion and alcoholysis [8]. The reactions are presented in Fig. 5.

The 2-chloroethylene ester was then introduced into a GC–ion trap [9]. The GC–ion trap was first tuned to meet DFTPP (decafluorotriphenylphosphine) criteria based on the US EPA requirement for pesticide analyses [10]. An injection of 8 ng of ethylene ester was found to be the adequate concentration in order to obtain the full spectrum of the molecular ion and its fragment ions. However, a double concentration in citrus was needed because of the complexity of matrix interferences in the citrus peels. An injection concentration of 1 to 2 ng was also

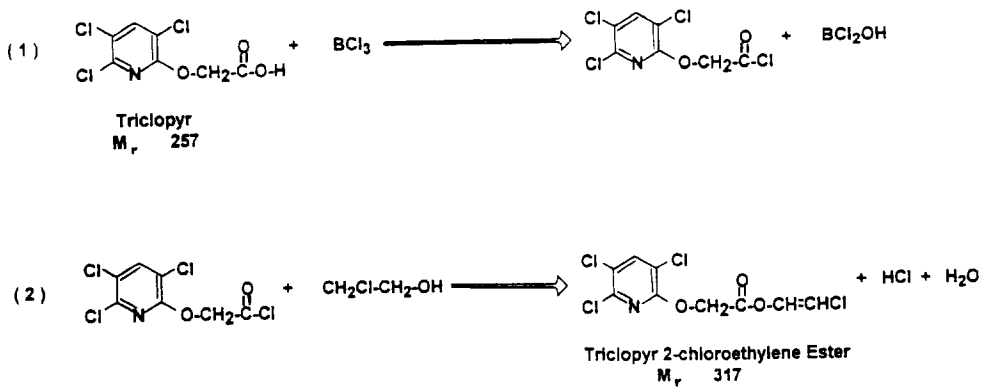

Fig. 5. (1) Acid chloride conversion, and (2) esterification through alcoholysis.

tested, the end result was that the isotopes' information around the major ions became unavailable.

The essence of MS in EI mode was shown in the spectra profile which provided significant information in terms of molecular structure [11]. The result was indisputable when the identification data were complemented with the injunction of elemental instrument analyses, such as the GC–ECD and the GC–NPD. Therefore, in order to distinguish this method, spectrum interpretation was the focal point of the study (Table 3). As mentioned before, 2-chloroethylene ester was a relatively stable compound in which the molecular ion ($m/z = 317$) could be found in the spectrum at the concentration level of 8 ng in

Table 3
MS interpretation of triclopyr 2-chloroethylene ester

Molecular ion	$m/z = 317$, $C_9H_5O_3NCl_4$				
Base ion	$m/z = 210$, $C_6H_2NOCl_3$				
Isotopes	Relative abundance				
	X	$X+2$	$X+4$	$X+6$	$X+8$
$m/z = 317$, Cl = 4	76.9	100	48.7	10.5	0.9
$m/z = 210$, Cl = 3	100	97.5	31.7	3.4	–
$m/z = 146$, Cl = 2	100	65.0	10.6	–	–
Appearance of characteristic ions	$m/z = 238$, $C_7H_2NO_2Cl_3$ $m/z = 210$, $C_6H_2NOCl_3$ $m/z = 182$, C_5HNCl_3 $m/z = 146$, C_5HNCl_2				
Nitrogen rule	Molecular weight = odd $m/z = 317$ Fragment ions = even mass $m/z = 238, 210, 182, 146$				

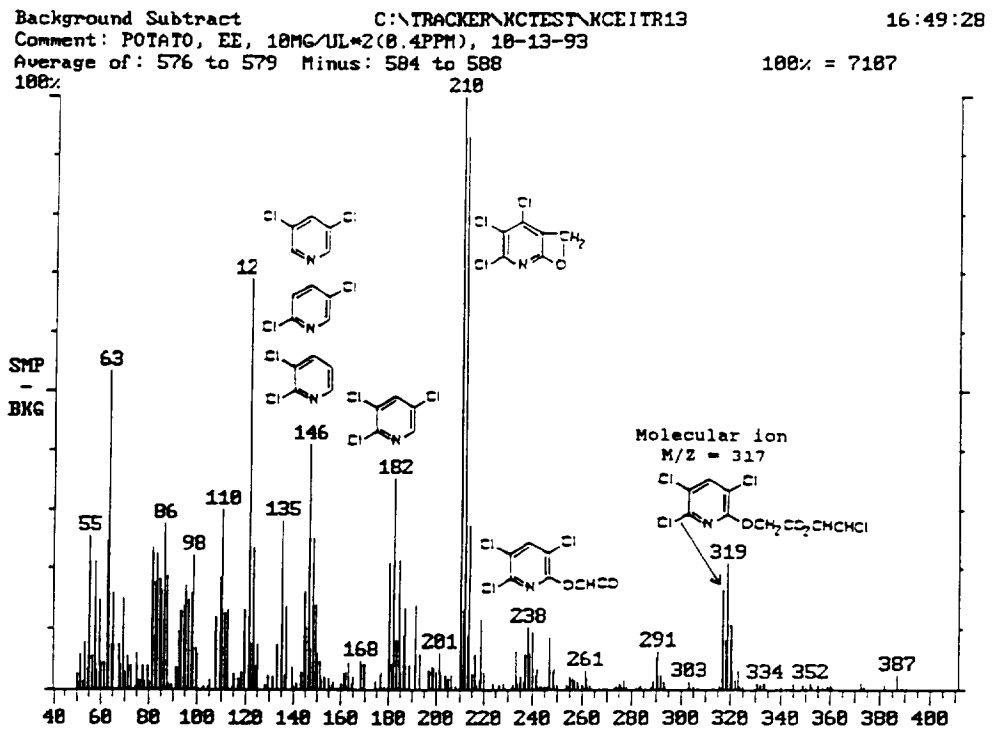

Fig. 6. Mass spectrum of triclopyr 2-chloroethylene ester in potato (0.4 ppm fortification).

Fig. 6. In the case of a synthesized compound like triclopyr 2-chloroethylene ester, the molecular ion information was very important as one can never be certain about the derivative process without this vital information. The base ion ($m/z = 210$) was the most abundant or frequently occurring ion in the profile because of the sigma-bond dissociation mechanism at a weakly bonded link between carbon and carboxyl, RC–COR', which, consequently, induced the McLafferty hydrogen atom rearrangement. Structurally, the base ion ($m/z = 210$) was $C_6H_2NOCl_3$ which was also the major ion used for methyl ester identification since the molecular ion was sometimes absent in the spectrum at the 8 ng concentration level. With a chlorinated compound, one of the unique displacements was the distribution of a isotope abundance ratio in the spectrum. For example, in Table 3, the molecular ion ($m/z = 317$) contained 4 chloride atoms, the isotope abundance ratios being $X = 76.9$, $X + 2 = 100$, $X + 4 = 48.7$, $X + 6 = 10.5$ and $X + 8 = 0.9$. In other examples, the base ion ($m/z = 210$) contained 3 chloride atoms, the isotope abundance ratios being $X = 100$, $X + 2 = 97.5$, $X + 4 = 31.7$ and $X + 6 = 3.4$ in Fig. 7, and the fragment ion ($m/z = 146$) had 2 chloride atoms, its isotope abundance ratios being $X = 100$, $X + 2 = 65.0$, and $X + 4 = 10.6$. These were excellent clues for a fingerprint match. In the fragmentation profile, there were character ions, such as $m/z = 238$, $C_7H_2NO_2Cl_3$; $m/z = 182$, C_5HNCl_3; and $m/z = 146$, C_5HNCl_2 and these were used as a means of identification in case of cleavage patterns. As mentioned before, the pyridine structure was a nitrogen containing compound; therefore, the nitrogen rule could be applied for more thorough identification. In this case, for example, the molecular weight should be odd and matched $m = 317$ (odd). Also, the fragment ions should have even mass, while the nitrogen containing fragment ions were $m/z = 238$, 182 and 146 (even). Again, these were great identifiers for the newly derivatized compound.

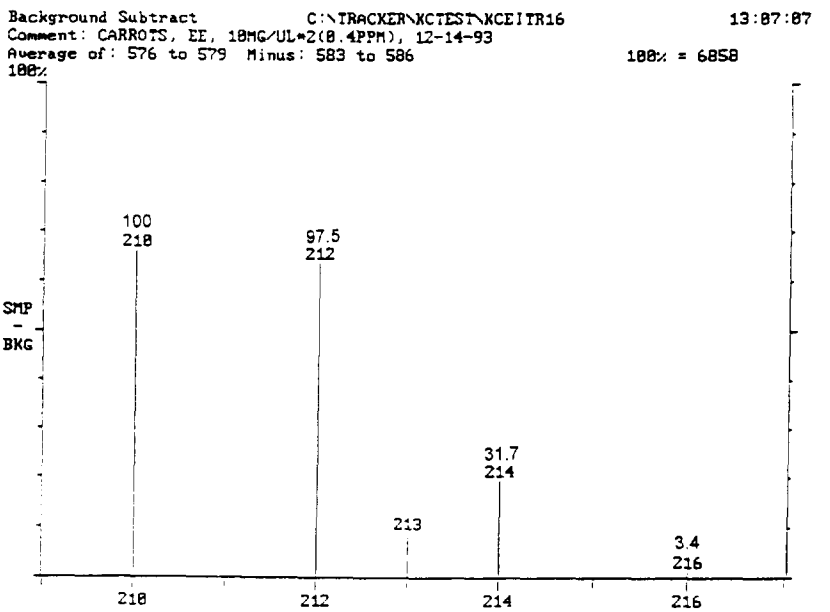

Fig. 7. Chlorine isotope peaks of $m/z = 210$ in the spectrum of carrots (0.4 ppm fortification).

4. Discussion

Structurally, triclopyr has many similarities to 2,4-D, 2,4,5-T and other phenoxyalkanoic acids. They are made up of aromatic rings (pyridine verses phenol) and carboxylic acid; and because of this, triclopyr had been analyzed like phenoxyacetic acids very successfully. However, sample preparation of the phenoxyacetic acid method is time consuming as it requires at least 6 hours processing time for a set of 2 to 3 samples per analyst without taking the GC analysis time into account. Furthermore, the method uses a large volume of solvents in the partition clean-up procedure and consequently, the disposal of liquid waste becomes an expensive process. In our method, we eliminated the partition steps prior to esterification and later used a micro-scale clean-up procedure that only required 5 ml water partition with hexane by repeating the step 3 times. This modification has reduced solvent waste almost to one-tenth and is a significant improvement in terms of decreasing waste and thus helps to protect the environment. In addition, one analyst could easily complete a set of 6 samples in 4 hours using our fast and simple sample preparation procedure.

Traditionally, diazomethane has been the single most popular derivatizing reagent for phenoxyacetic acid analyses [6]. However, because of its highly unstable and volatile nature, fresh diazomethane must be prepared for each batch of sample analyses. Long term storage is not recommended due to the problem of degradation. In addition, because diazomethane is both explosive and exceedingly toxic, it causes much fear and anxiety among analysts during its usage in laboratories. Analytically, diazomethane reacts with carboxylic acid to form methyl ester which has a short retention time under usual GC conditions. This is an unfortunate situation as interferences from sample co-extractives generally appear in the early region of the chromatogram.

Keeping all the diazomethane associated deficiencies in mind, 2-chloroethanol–BCl_3 is an obvious replacement in this line of research [12]. 2-Chloroethanol–BCl_3 is a non-explosive, and less toxic chemical than diazomethane. The reagent is capable of enhancing the halogen

detector response due to an extra chloride atom added to the original compound. Furthermore, the added chloride moiety increases the derivative compound's polarity or electronegativity which in turn prolongs retention time in the GC column and leads to better separation and resolution. In addition, 2-chloroethanol derivative is a better compound for GC–MS analysis as it is more stable than methyl ester at the ion source. In other words, some molecular ions could survive the ion beam bombardment without undergoing fragmentation and ultimately reach the detector intact. Because the mass ion could then be found in the spectrum, identification is simpler and faster.

Although 2-chloroethanol–BCl_3 has many advantages, one should also be aware of its weaknesses. In comparison to diazomethane, 2-chloroethanol–BCl_3 contributes more background noise in the halogen detector. During the preliminary trial, the reagent was first washed with hexane before use and then incorporated with 5 ml of NaOH (5 *M*) after esterification in the partition clean-up step so as to neutralize excessive chlorides [12]. These treatments proved to be insignificant as there was no noticeable improvement. For this reason, combined GC–MS identification and GC quantitation is a crucial step in obtaining quality data.

5. Conclusion

Triclopyr residue in fruits and vegetables is analyzed like other phenoxyalkanoic acids; the current method is time consuming, needs a large amount of solvents, and involves a dangerous chemical, diazomethane, as a derivatizing reagent.

In this research, the entire solvent volume used for analysis was reduced to one-tenth of that used in the existing method. Diazomethane was replace by 2-chloroethanol–BCl_3, a stable and safe reagent. In order to overcome the identification or qualitative difficulty which was caused by 2-chloroethanol–BCl_3 background noise, the multiple instrumentation approach was taken to assure a high standard of quantitative and qualitative data. The results generated by these experiments demonstrate that this method is simple, fast, safe and reliable.

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