Chapter 2

Development of A Capillary Gas Chromatographic Method for Estimation of Active Ingredients and Isomeric Contents of Several Pesticides in Various Commercial Products Using Suitable Internal Standards

CONTENTS

1. Introduction

Pesticides are the essential components of modern agricultural production. As a plant protection tool pesticides have boosted food production manifold in the last 40 years. But their indiscriminate use has lead to wide spread contamination of the environment, i.e. soil, water, air and food. Therefore their careful and judicious use is necessary. The efficiency of pesticide application is the greatest when the biggest fraction of applied dose is collected by specified biological target. It is only possible when appropriate application technology is used and active ingredient content and isomeric content analysis of various technical and formulated pesticides have been conducted appropriately.

Several classical and instrumental methods have been published in CIPAC Handbook[1], Official Methods of AOAC[2], BIS[3], FAO[4] and various books[5] on analyses to evaluate the pesticide quality in various technical and formulated materials. Majority of the methods employed either a gas chromatograph with flame ionization detector (GC-FID) or a high performance liquid chromatograph with UV detector (HPLC-UV) for estimation of active ingredient or isomeric content of single pesticide using a suitable internal standard. Therefore, It is not easy task to analyse the different pesticide contents in various commercial products by changing instruments, columns and conditions again and again.

An attempt has been made to develop a simple and efficient gas chromatographic method to estimate the active ingredients and isomeric contents of twenty chlorinated pesticides using suitable internal standards. The proposed capillary gas chromatograph equipped with flame ionization detector (GC-FID) was useful for estimation of active ingredient contents of heptachlor, aldrin, dieldrin, alachlor, trifluralin, bifenthrin, hexachlorobenzene and hexaconazole in various commercially available technical and formulated pesticides. The method was also suitable for evaluation of isomeric ratios of various pesticides like, DDT, HCH, endosulfan, chlorothalonil, lambda-cyhalothrin, permethrin, cypermethrin, fenvalerate and deltamethrin using suitable internal standards.

2. Experimental Procedure

2.1 Instruments and Equipments

Sr. No.	Instruments	Model	Manufacturer
1.	Weighing Balance (Least Count 0.01 mg)	CP 225 D	Sartorius, Germany
2.	Gas Chromatograph with Flame Ionization Detector	Star 3350	Varian, USA
3.	GLC Column [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]	DB-5	SGE, Australia

2.2 Solvents and Chemicals

Sr. No.	Solvents/Reagents	Grade	Supplier
1.	n-hexane	ExcelaR	Qualigens, India

2.3 Internal Standards

Sr. No.	Chemicals	Purity (%)	Supplier
1.	1-Naphthol	99.0	Qualigens, India
2.	Triphenyl methane	99.0	Merck-Schuchardt, Germany
3.	4,4-dimethoxy benzophenone	99.0	s. d. fine chem. Limited, India
4.	Dicyclohexyl phthalate	>99.0	Merck-Schuchardt, Germany
5.	Bis (2-ethylhexyl phthalate)	98.0	s. d. fine chem. Limited, India
6.	Octacosane	99.0	Sigma-Aldrich, USA
7.	Dibutyl phthalate	>99.0	Qualigens, India
8.	Diethyl phthalate	99.0	Merck-Schuchardt, Germany
9.	Triphenyl benzene	98.0	Qualigens, India

2.4 Reference Standards

Sr. No.	Pesticides	Purity (% w/w)	Source
1.	α-HCH (BHC)	99.3	Chem Service, USA
2.	γ-HCH (Lindane)	99.0	Chem Service, USA
3.	Trifluralin	99.7	Chem Service, USA
4.	Heptachlor	99.1	Chem Service, USA
5.	Aldrin	98.6	Chem Service, USA
6.	Dieldrin	98.7	Chem Service, USA
7.	Endrin	99.5	Chem Service, USA
8.	Alachlor	96.0	Chem Service, USA
9.	Butachlor	98.9	Chem Service, USA
10.	Endosulfan-I	99.5	Chem Service, USA
11.	Endosulfan-II	99.2	Chem Service, USA
12.	DDT (p,p')	99.1	Chem Service, USA
13.	Bifenthrin	98.0	Chem Service, USA
14.	λ-cyhalothrin	98.0	Chem Service, USA
15.	Permethrin	Cis: 44.0 Trans: 55.0	Chem Service, USA
16.	Cypermethrin (Mix. of Isomers)	98.0	Chem Service, USA
17.	Fenvalerate	98.3	Jai Research Foundation, India
18.	Deltamethrin	99.9	Jai Research Foundation, India
19.	p-Chlorothalonil	95.2	Sigma-Aldrich, USA
20.	m-Chlorothalonil	99.2	Sigma-Aldrich, USA
21.	o-Chlorothalonil	99.77	Sigma-Aldrich, USA
22.	Hexaconazole	99.0	Chem Service, USA
23.	Hexachlorobenzene	99.87	Sigma-Aldrich, USA

2.5 Pesticide Commercial Samples

Sr. No.	Pesticides	Type of Product	Purity (%)	Source
1.	α-HCH	Technical	97.2	M/S B. V. Industrie- & Handelsonderneming Simonis, Holand
2.	Lindane	20% EC	20.3	M/S Krishi Rasayan Exports Pvt. Ltd.,India
3.	Trifluralin	Technical	96.3	M/S King Tech Corp., China
4.	Endosulfan	35% EC	35.5	M/S Agrolex Pte. Ltd. India
5.	Heptachlor	Technical	97.1	M/S Krishi Rasayan Exports Pvt. Ltd., India.
6.	Butachlor	50% EC	49.8	M/S Bharat Rasayan Ltd., India
7.	Alachlor	10% GR	10.1	M/S B. V. Industrie- & Handelsonderneming Simonis, Holand
8.	DDT	Technical	97.5	M/S King Tech Corp. China
9.	Aldrin	Technical	96.4	M/S B. V. Industrie- & Handelsonderneming Simonis, Holand
10.	Dieldrin	Technical	96.9	M/S King Tech Corp., China
11.	Endrin	Technical	97.2	M/S B. V. Industrie- & Handelsonderneming Simonis, Holand
12.	Bifenthrin	Technical	96.8	M/S United Phosphorus Limited, India
13.	λ-cyhalothrin	Technical	95.2	M/S Agro Chemicals Industries Ltd., India
14.	Cypermethrin	25% EC	25.3	M/S Indofil Chemicals Co., India
15.	Permethrin	Technical	97.5	Agro Chemicals Industries Ltd., India
16.	Fenvalerate	20% EC	19.9	United Phosphorus Limited, India
17.	Deltamethrin	2.5% WP	2.5	Heranba Industries Limited, India
18.	Hexaconazole	5% EC	5.1	United Phosphorus Limited, India
19.	Chlorothalonil	Technical	97.8	Agro Chemicals Industries Ltd., India
20.	Hexachloro= benzene	Technical	96.9	Krishi Rasayan Exports Pvt. Ltd., India

2.6 Methodology

The active ingredient content analysis of commercial samples was performed using internal standard method by gas chromatography coupled with flame ionization detector and a capillary column. A known concentration solution of each commercial sample was prepared using suitable internal standard. The standard solution of one or other reference standards was prepared using suitable internal standards for simultaneous analysis of various active ingredient contents in commercial samples. Sample solutions of formulated compounds were filtered through whatman filter paper 42, before injection onto GC.

2.6.1 Internal Standard Stock Solutions

A known quantity of each internal standard (1.0 g, approx.) was weighed into separate volumetric flasks of 100 mL capacity, contents were dissolved in 50 mL n-hexane and volume was made upto the mark with n-hexane (concentration 10,000 ppm for each internal standard).

2.6.2 Reference Standard Solutions with Internal Standard

The pesticide standards were homogenized by proper mixing before standard solutions preparation. Semi-liquid standards viz., cypermethrin, permethrin, fenvalerate were heated gently to liquify and stirred to homogenize. A known quantity of each reference standard (10 mg, approx.) was weighed into separate volumetric flasks of 10 mL capacity, 1 mL internal standard stock solution was added to dissolve the material and volume was made upto the mark with n-hexane (1000 ppm concentration of both reference standard and internal standard).

2.6.3 Standard Mixture of Reference Standards and Internal Standards

A known quantity of each reference standard (10 mg, approx.) was weighed into a volumetric flask of 10 mL capacity, a volume of 1 mL internal standard stock solution was added into same volumetric flask and volume was made upto the mark with n-hexane (1000 ppm concentration of each reference standard and internal standard).

2.6.4 Sample Solutions with Internal Standard

The commercial samples were homogenized by proper stirring, before sample solution preparation. Semi-liquid samples were heated gently to liquify and stirred to homogenize. A known quantity of each homogenized sample (50 mg content, approx.) was weighed in duplicate into separate volumetric flasks of 50 mL capacity, 5 mL internal standard stock solution was added to dissolve the material and volume was made upto the mark with n-hexane (approx. 1000 ppm concentration of active ingredient and internal standard). Sample solutions were filtered through whatman filter paper 42, if required. Each sample and standard solution was injected onto GLC in triplicate and active ingredient contents were calculated by comparing the relative sample response with mean relative standard response. The standard and sample solutions were stored in refrigerator.

2.7 Gas Chromatographic Conditions

The standard solutions and sample solutions were analysed by a gas chromatograph equipped with flame ionization detector (GC-FID) using following parameters:

Instrument	:	Gas Chromatograph, Varian (3350) with Star Workstation Data System
Column	:	DB-5, [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]
Oven temp.	:	180 °C (hold for 1 min) to 280 °C @ 8 °C/min (hold for 4 min)
Injector temp.	:	260 °C
Detector temp.	:	280 °C
Detector	:	Flame Ionization Detector (FID)
Injection mode	:	Split (20 : 1)
Injection volume	:	1 µl
Carrier gas	:	Nitrogen
Carrier gas flow	:	1.5 mL/min
Make-up gas flow	:	30 mL/min
Hydrogen flow	:	30 mL/min
Air flow	:	300 mL/min
Retention Time	:	**(Refer Table 1)**

Table 1: Retention times of pesticides and their internal standards analysed by GLC.

Sr. N°	Pesticide	Retention Time (min)	Internal Standard	Retention Time (min)
1.	α-HCH	2.48	1-Naphthol	1.68
2.	γ-HCH (Lindane)	2.82		
3.	Trifluralin	2.22	Dibutyl phthalate	4.03
4.	Hexachlorobenzene	2.58		
5.	Heptachlor	3.73	Triphenyl methane	4.49
6.	Aldrin	4.25	Triphenyl methane	4.49
7.	Dieldrin	6.05	4,4-dimethoxy benzophenone	6.62
8.	Endrin	8.51	Dicyclohexyl phthalate	9.17
9.	Alachlor	3.64	Triphenyl methane	4.49
10.	Butachlor	5.62	4,4-dimethoxy benzophenone	6.62
11.	Endosulfan-I	5.53	Triphenyl methane	4.49
12.	Endosulfan-II	6.64		
13.	DDT (p,p')	7.60	Dicyclohexyl phthalate	9.17
14.	Bifenthrin	8.79	Bis (2-ethylhexyl phthalate)	9.44
15.	Lambda-cyhalothrin-I	9.80	Dicyclohexyl phthalate	9.17
16.	Lambda-cyhalothrin-II	10.04		
17.	Permethrin-I	10.85	Octacosane	11.75
18.	Permethrin-II	11.02		
19.	Cypermethrin	12.14	Bis (2-ethylhexyl phthalate)	9.44
20.	Fenvalerate-I	13.11	Octacosane	11.75
21.	Fenvalerate-II	13.37		
22.	Deltamethrin	14.08	Triphenyl benzene	13.01
23.	p-Chlorothalonil	3.08	Dibutyl phthalate	4.03
24.	m-Chlorothalonil	3.19		
25.	o-Chlorothalonil	3.40		
26.	Hexaconazole	5.82	4,4-dimethoxy benzophenone	6.62

2.8 Sample Analysis

The repeated injections of 1 µL of the standard solution were made onto a gas liquid chromatograph operated under the conditions described in **section 2.7** to obtain a consistent response (± 1%). Consecutive injections of standard and sample solutions were made in the sequence: standard, standard, sample-1, sample-1, standard, sample-2, sample-2, standard, standard. The pesticides active ingredient contents and isomeric contents were calculated using internal standard method by comparing the sample relative response with standard average relative response, using the formula provided below.

2.9 Calculation for Internal Standard Method

$$RF' = \frac{A_r'}{A_i'}; \qquad RF = \frac{A_s}{A_i}$$

where,

RF'	=	Relative response factor for reference standard
RF	=	Relative response factor for sample
A_i'	=	Area of internal standard peak in standard solution
A_r'	=	Area of active ingredient / isomeric peak in standard solution
A_i	=	Area of internal standard peak in sample solution
A_s	=	Area of active ingredient / isomeric peak in sample solution

$$\text{Active ingredient / isomeric content (\% w/w)} = \frac{RF \times W' \times P}{RF'_{ave} \times W}$$

where,

RF'_{ave}	=	Average relative response factor for standard
RF	=	Relative response factor for sample
W'	=	Weight (mg) of standard
W	=	Weight (mg) of sample
P	=	Purity of reference standard (% w/w)

3. Results and Discussion

The capillary gas chromatographic method with flame ionization detector was efficient to separate the active ingredients and isomers of more than thirteen chlorinated pesticides within 15 min (**Fig. 1**).

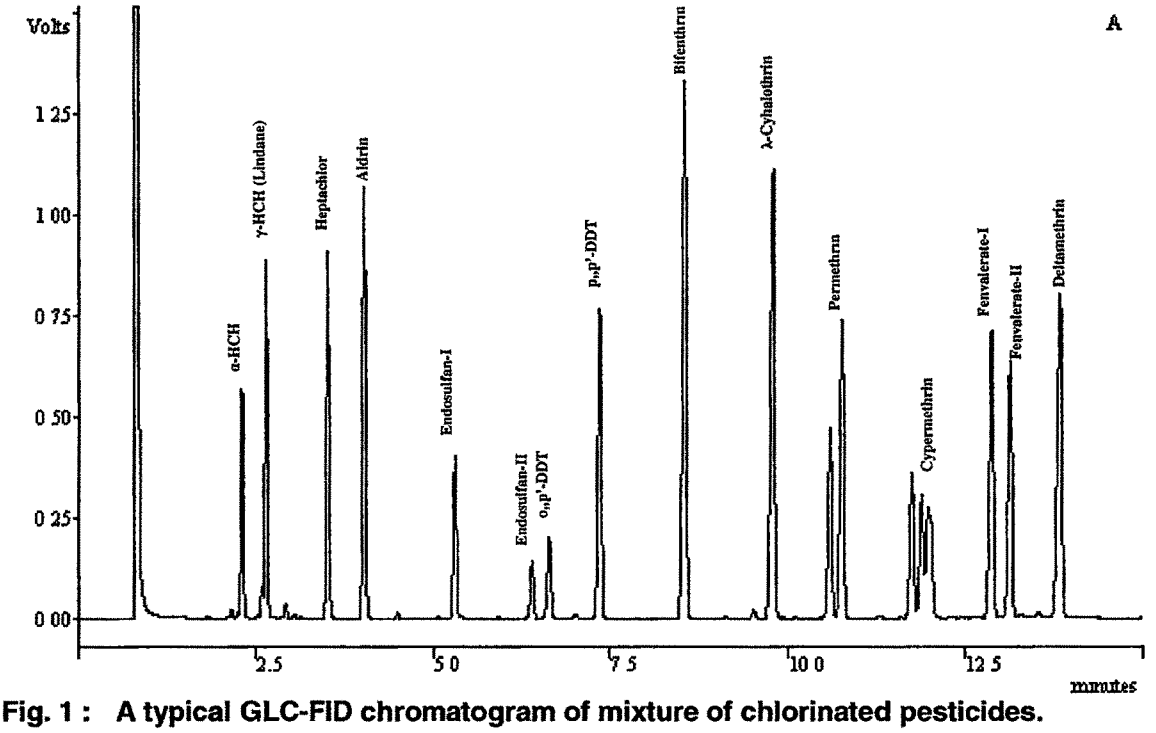

Fig. 1 : A typical GLC-FID chromatogram of mixture of chlorinated pesticides.

The active ingredient contents of commercial pesticide samples were determined by duplicate sample preparation of each product using internal standard and injecting each sample thrice along with standard solution onto gas chromatograph. The active ingredient contents were calculated by comparing the relative responses of analyte to internal standard in sample and standards. The method was very useful for simultaneous estimation of several active ingredients present in different commercial pesticide products using suitable internal standards (**Table 1**). **Fig. 2** shows the simultaneous analysis of butachlor and hexaconazole using 4,4-dimethoxy benzophenon as internal standard. Similarly alachlor and aldrin was analysed with

triphenyl methane as internal standard (**Fig. 3**); and lambda-cyhalothrin and DDT with dicyclohexyl phthalate as internal standard (**Fig. 4**).

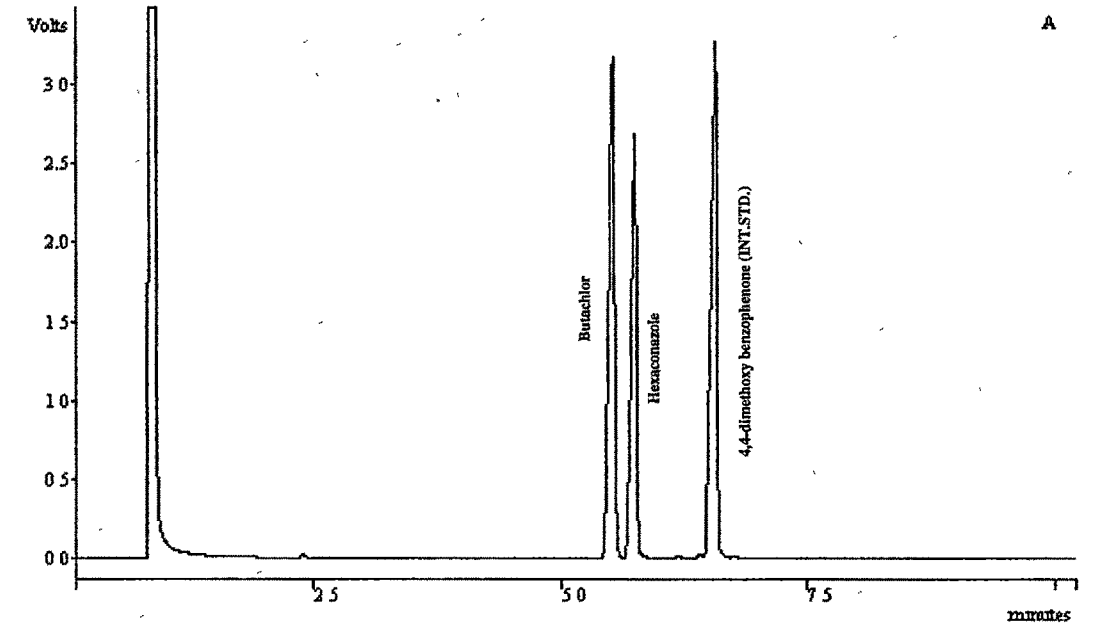

Fig. 2 : Gas chromatogram for simultaneous analysis of butachlor and hexaconazole technical pesticides using 4,4-dimethoxy bezophenone internal standard.

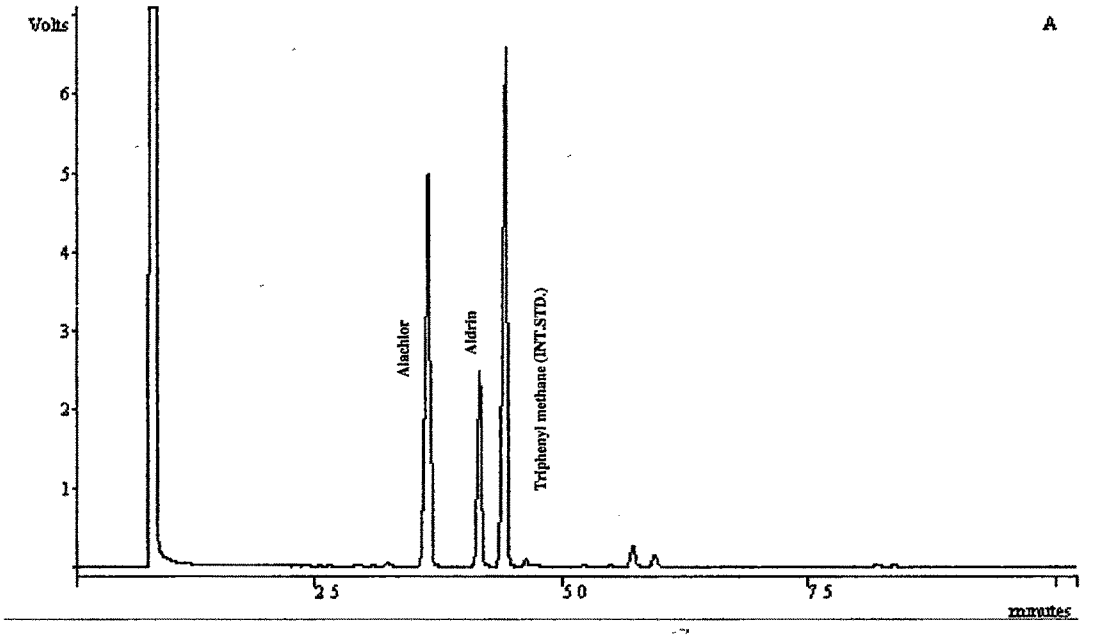

Fig. 2 : Gas chromatogram for simultaneous analysis of alachlor and aldrin technical pesticides using triphenyl methane internal standard.

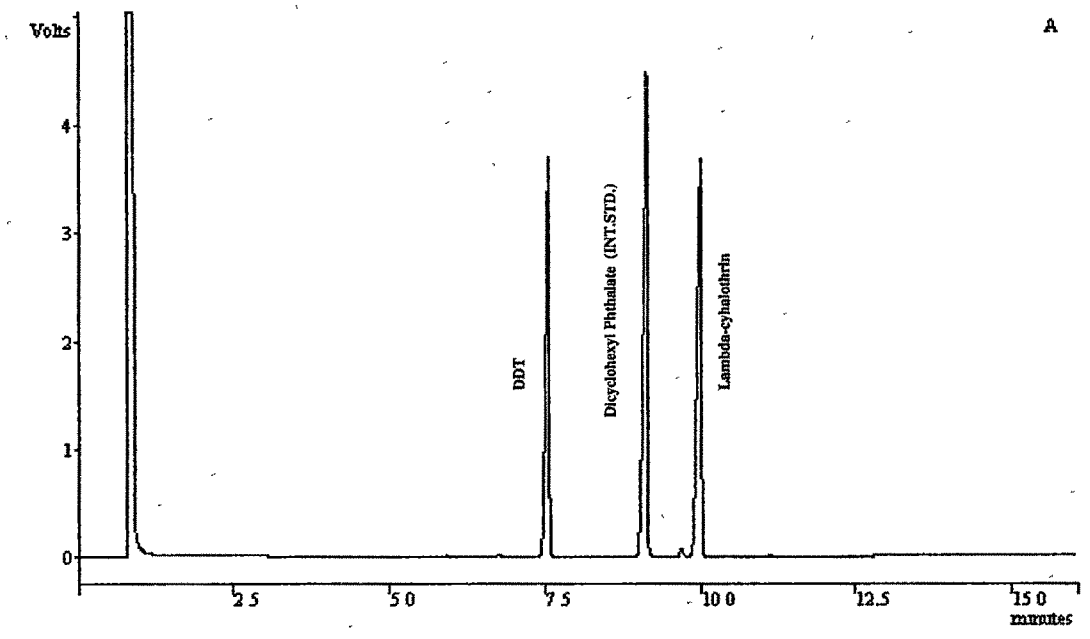

Fig. 3 :Gas chromatogram for simultaneous analysis of DDT and lambda-cyhalothrin pesticides using dicyclohexyl phthalate internal standard.

The proposed GLC-FID method was also suitable for isomeric contents analysis of various pesticides in commercial products. Ortho-, para- and meta-isomers of chlorothalonil were analysed with dibutyl phthalate internal standard (**Fig. 4**). Similarly, the isomers of fenvalerate and permethrin were efficiently separated and analysed with octacosane as intenal standard (**Fig. 5 & 6**), while isomers of deltamethrin and cypermethrin were analysed using trimethyl benzene and bis (2-ethylhexyl phthalate) as internal standards, respectively (**Fig. 7 & 8**). Various isomers of hexachlorocyclohexane (HCH) and Endosulfan were also quantified successfully using proposed method (**Fig. 9, 10**).

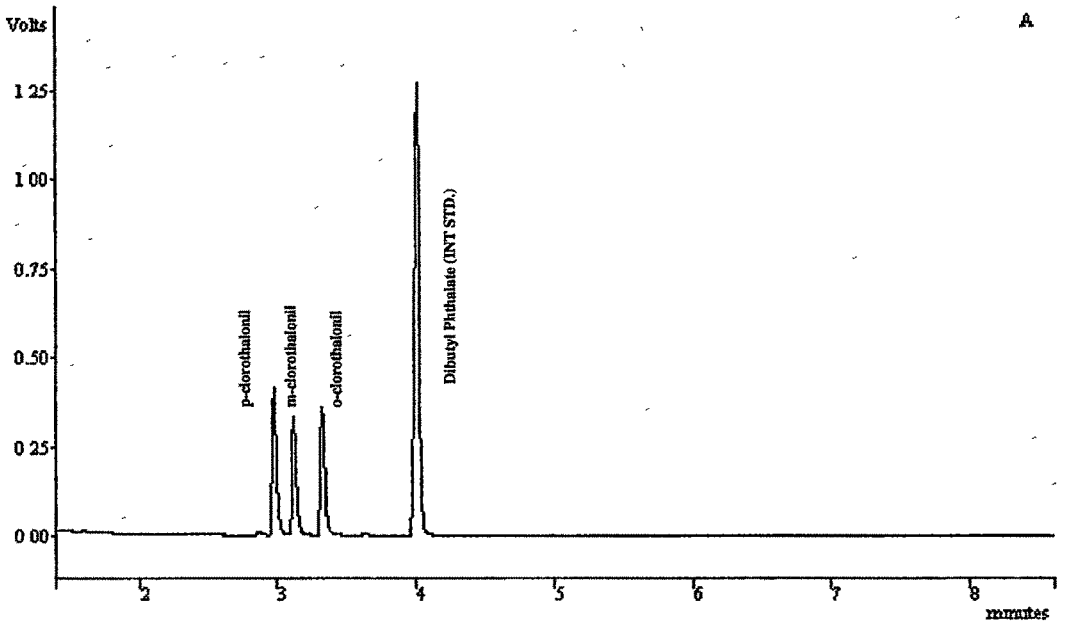

Fig. 4 :Gas chromatogram for simultaneous analysis of isomers of chlorothalonil using dibutyl phthalate internal standard.

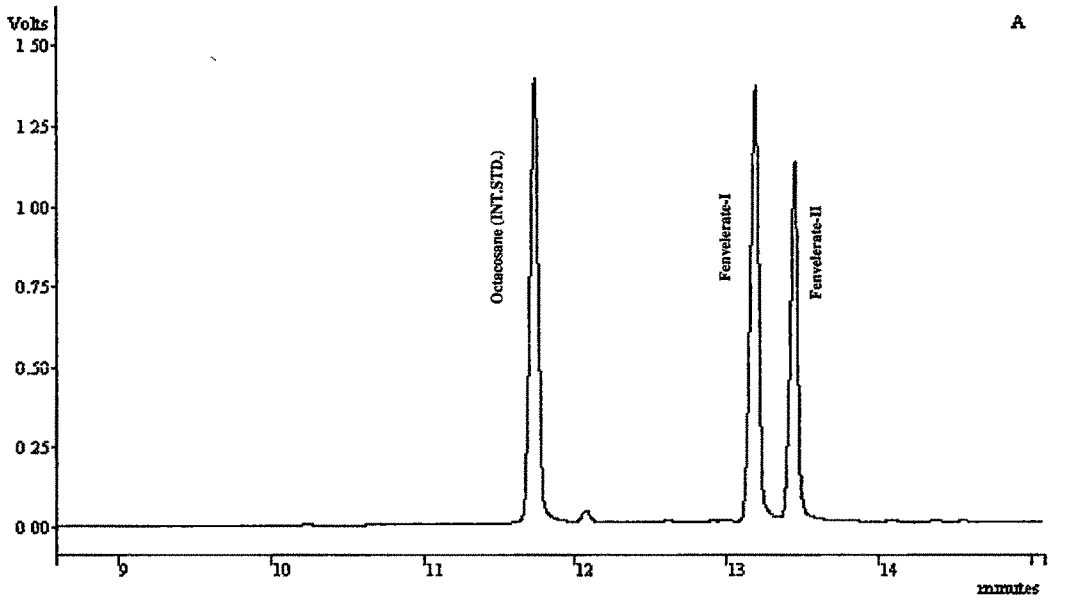

Fig. 5 :Gas chromatogram for simultaneous analysis of isomers of fenvalerate using octacosane internal standard.

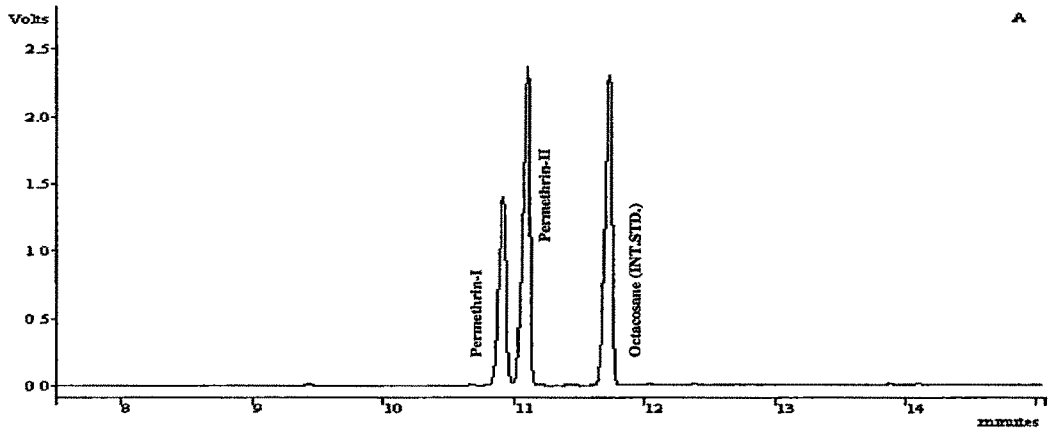

Fig. 6 :Gas chromatogram for simultaneous analysis of isomers of permethrin using octacosane internal standard.

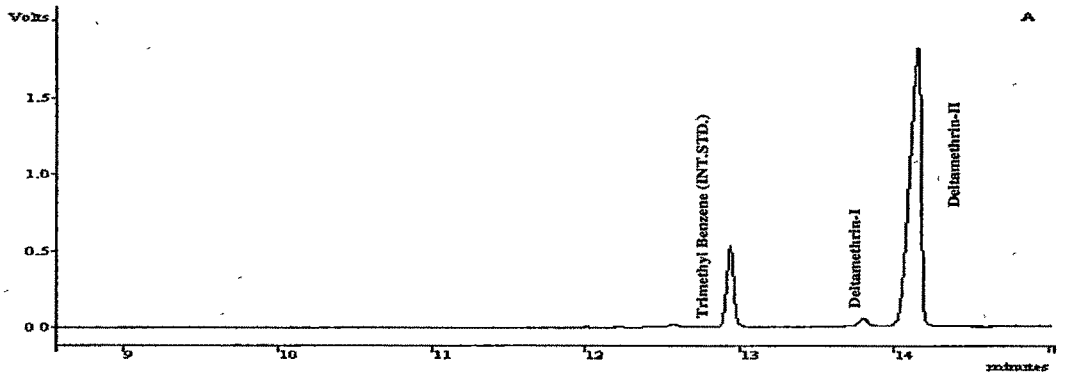

Fig. 7 :Gas chromatogram for simultaneous analysis of isomers of deltamethrin using trimethyl benzene internal standard.

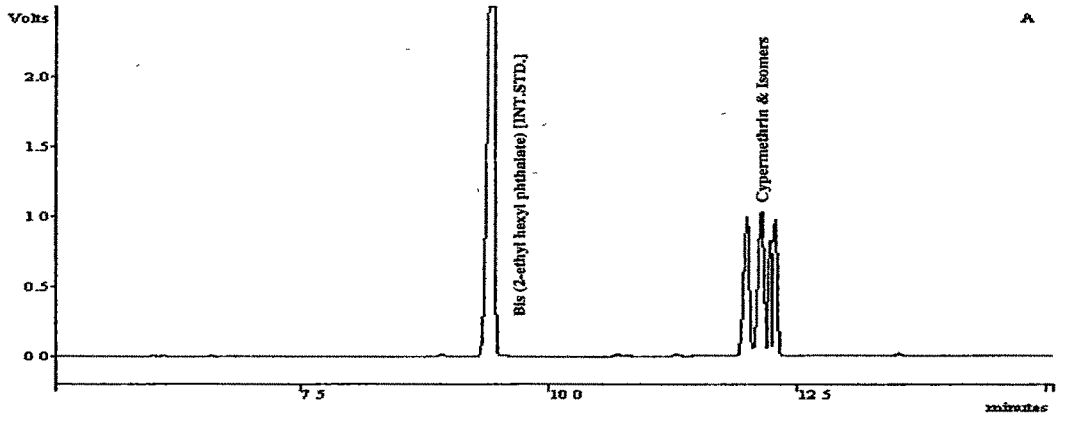

Fig. 8 :Gas chromatogram for simultaneous analysis of isomers of cypermethrin using bis-(2-ethylhexyl phthalate) internal standard.

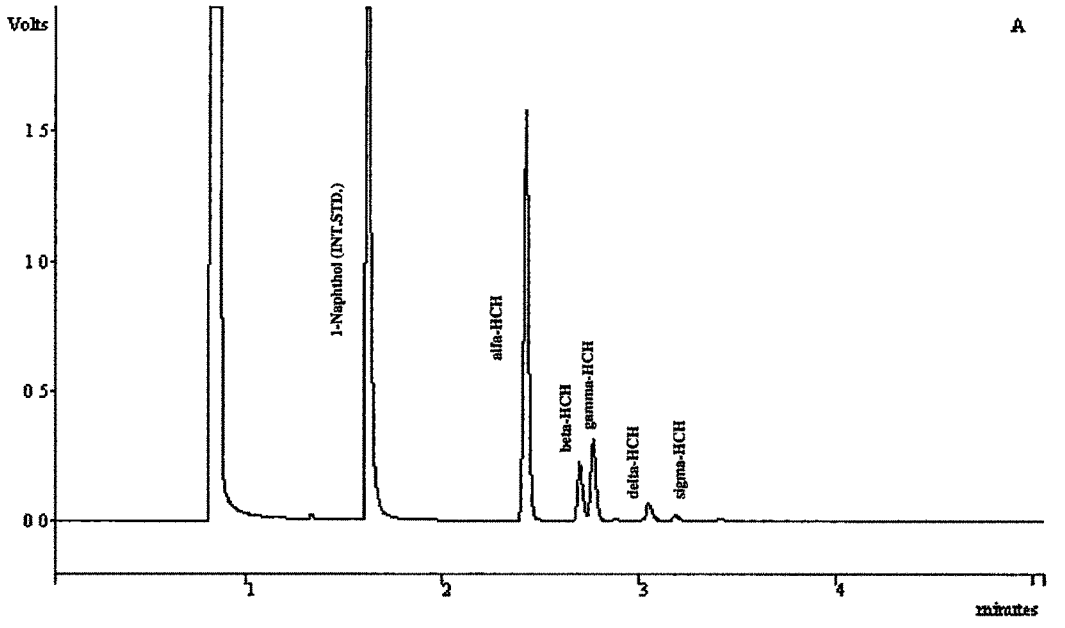

Fig. 9 :Gas chromatogram for simultaneous analysis of isomers of hexachloro= cyclohexane (HCH) using 1-Naphthol as internal standard.

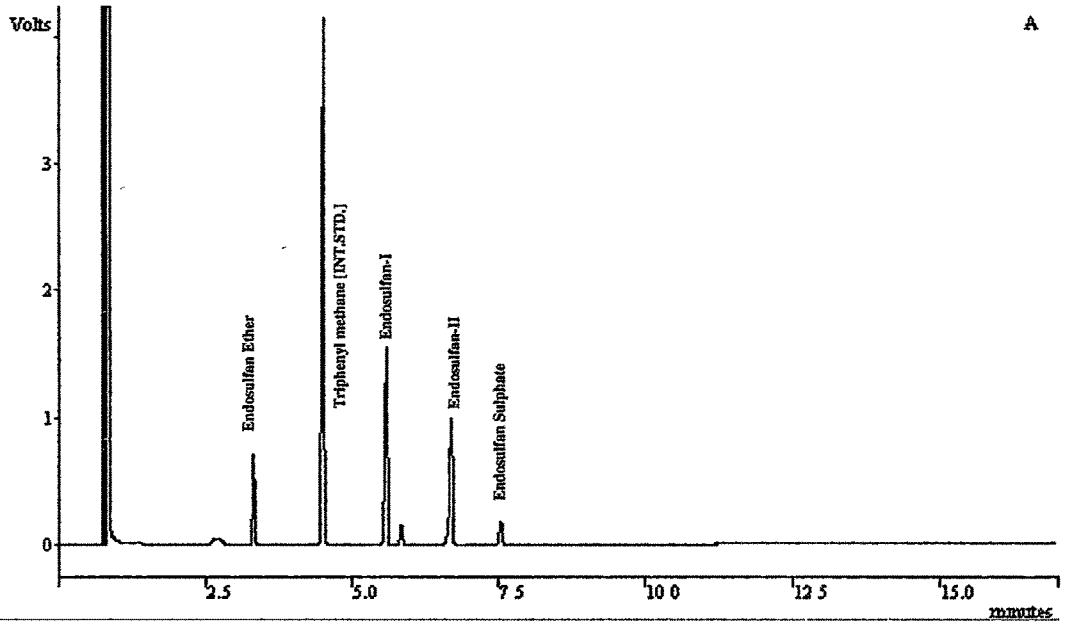

Fig. 10 : Gas chromatogram for simultaneous analysis of isomers of endosulfan and associated impurities using triphenyl methane as internal standard.

The capillary gas chromatographic method was validated before analyzing the active ingredient contents of different pesticides. The method validation covered the aspects viz., (i) specificity, (ii) linear dynamic range (LDR), (iii) limit of detection (LOD), (iv) limit of quantitation (LOQ), (v) precision (% RSD) [repeatability/reproducibility] and (vi) accuracy (% recovery). The correlation co-efficients (r) of pesticides for concentration range of 5 ppm to 5000 ppm were in the range of 0.9986 to 0.9999. The limit of detection (LOD) of the method (signal to noise ratio, S/N of 3 ± 0.5 : 1) ranged between 0.1 to 0.5 ppm and limit of quantitation (LOQ) [S/N ratio between 5 : 1 to 10 : 1] varied from 0.2 to 1.0 ppm. The relative standard deviation (% RSD) for five replicate injections of technical grade pesticide samples was between 0.82 to 1.18%. The accuracy of method was determined using standard addition method by fortifying the known quantity of standards at three fortification levels into the samples and calculating the recoveries using internal standard method. The mean % recovery for various active ingredients varied from 96.5% to 102.1%.

Table 2: Method Validation Data of pesticides with GLC-FID.

Sr. N°	Compound	Limit of Detection (ppm)	Limit of Quantitation (ppm)	Regression Co-efficient (r)	Relative Standard Deviation (% RSD)	Average Recovery (%)
1.	HCH	0.2	0.5	0.9997	0.82	96.5
2.	γ-HCH (Lindane)	0.1	0.2	0.9995	0 95	99.4
3.	Heptachlor	0.2	0.5	0.9999	0.98	102.1
4.	Aldrin	0.1	0.2	0.9997	1.12	98.8
5.	Endosulfan-I	0.2	0.5	0.9989	0.89	101.6
6.	Endosulfan-II	0.5	1.0	0.9991	0.97	99.1
7.	p,p'-DDT	0.5	1.0	0.9995	1.04	97.6
8.	Bifenthrin	0.1	0.2	0.9996	0.87	98.9
9.	λ-cyhalothrin	0.2	0.5	0.9998	0.93	99.5
10.	Permethrin-I	0.2	0.5	0.9986	1.15	96.8
11.	Permethrin-II	0.2	0.5	0.9988	0.85	97.5
12.	Fenvalerate-I	0.1	0.2	0.9993	0.95	100.2
13.	Fenvalerate-II	0.2	0.5	0.9989	1.09	99.7
14.	Deltamethrin	0.5	1.0	0.9999	1.18	100.8

4. Conclusion

A capillary gas chromatographic method with flame ionization detector was developed for fast estimation of several active ingredients present in different commercial products (technical and formulations) using internal standards. The method was suitable to analyse the active ingredients of various pesticides viz., lindane, DDT, aldrin, endrin, dieldrin, heptachlor, alachlor, butachlor, bifenthrin, hexaconazole and separation as well as quantification of isomeric contents of various pesticides viz., endosulfan, HCH, chlorothalonil, lambda-cyhalothrin, permethrin, cypermethrin, fenvalerate and deltamethrin. The proposed GLC method was validated for analyzing various pesticides. The regression co-efficient values were in the range of 0.9986 to 0.9999, % RSD for five repeatable injections was between 0.82 to 1.18% and percentage recoveries for various pesticides varied from 96.5% to 102.1%. Therefore, the proposed method is useful for routine estimation of active ingredients and isomeric contents of more than twenty chlorinated pesticides in various commercial pesticide products.

5. References

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