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Monitoring of Organochlorine Pesticide Residues in Fruits and Vegetables by using Gas Chromatography

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Abstract

Twenty organochlorine pesticides (OCPs) were monitored using gas chromatography-electron capture detector (GC-ECD) technology in five fruits (apple, pomegranate, litchi, plum, and papaya) and five vegetables (bottle gourd, ridge gourd, pointed gourd, bitter gourd, and brinjal). The results of the study indicated that two pesticides α-BHC and δ-BHC pesticides were present in the apple sample, three pesticides δ-BHC, heptachlor epoxide, and dieldrin pesticides were present in the pomegranate sample, six pesticides δ-BHC, heptachlor epoxide, α-chlordane, endosulfan I, dieldrin, and endrin ketone were found in the litchi sample, three pesticides heptachlor, heptachlor epoxide, and endrin ketone were found in plums sample, and two pesticides, δ-BHC, and heptachlor epoxide were found in papaya sample; heptachlor epoxide was found in bottle gourd and ridge gourd, and δ-BHC and heptachlor epoxide pesticides were present in pointed gourd sample, two pesticides δ-BHC and Heptachlor epoxide were found in bitter gourd, and Heptachlor epoxide was found in brinjal. Among all the pesticides obtained, the residue value of heptachlor epoxide was found to be higher. However, the residue values of all those pesticides were below the maximum residue limits (MRLs) values but their continued consumption may cause acute or long-term risk in humans. Therefore, it is necessary to continuously monitor pesticide residues in fruits and vegetables.

***Key words*:** Organochlorine pesticides, Monitor, Fruits, Vegetables, Gas chromatography

Pesticides are widely used to prevent or destroy pests in houses and agriculture [1]. However, the widespread use of pesticides in foods has led to the appearance of harmful pesticide residues in foods that can pose potential health risks to humans [2]. The healthy food pyramid also recommends eating fruits and vegetables for a variety of reasons, including the prevention of various diseases and low-calorie intake [3]. A higher intake of fruits and vegetables has been associated with a better quality of human health, including lower risks of cancer [4], malignant growth [5], heart disease [6], chronic diseases [7], Parkinson's [8], Alzheimer's [9], and poor health such as malnutrition [10], micro-nutritional deficiencies [11], overweight, and obesity [12-13] etc. Multiple residues of pesticides remain in fruits and vegetables. Therefore, it is essential to carry out regular and comprehensive testing of fruits and vegetables by regulatory administrative organizations to ensure that the concentration levels of toxic pesticides in fruits and vegetables will remain below tolerance levels [14-15].

India is one of the biggest manufacturers overall and is the second biggest producer of pesticides in Asia [16]. There has been a successive rise in the production and utilization of pesticides in India during the most recent thirty years. The utilization pattern of pesticides differs from the rest of the world, as in India, 76% of absolute pesticide utilization is insecticide as against 44% around the world of the total pesticide use, 45% goes to cotton crops followed by paddy and wheat. While just 10-12% of total pesticides are utilized for fruits and vegetables [17-18].

In recent years, the use of pesticides has increased to meet food demand through agricultural technology [19]. The levels of pesticide residues and their chemical properties in food items are an important problem that directly affects food production. It affects human health worldwide. Pesticides in food and in the environment have been shown to influence genetic polymorphisms and cause disease development [20].

Organochlorine pesticides are of significant concern worldwide due to their eco-toxicological properties. However, organochlorine pesticides have been banned in many European and Asian countries, yet due to their low cost and activity against a wide variety of organisms, insecticides including DDT, HCH, aldrin, and dieldrin are used in most Asian developing countries [21].

This paper deals with monitoring of concentration level of organochlorine pesticides (OCPs) in five fruits (apple, pomegranate, litchi, plum, and papaya) and five vegetables (bottle gourd, ridge gourd, pointed gourd, bitter gourd, and brinjal) purchased from the local market of Agra. In this monitoring, we have used GC using an ECD because this method is a reliable and selective method for the determination and detection of a wide range of organochlorine pesticides at trace levels. Knowing the number of organochlorine pesticides in fruits and vegetables, we can quickly analyze the potential

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health risks to humans after consuming those vegetables and fruits.

MATERIALS AND METHODS

Every glassware was washed with deionized water and dried in an oven at 150 °C. Before usage, all solvents were distilled. Before use, adsorbents such as neutral charcoal, florisil, and alumina were activated. Purified extracts of fruits and vegetables were analyzed using gas chromatography with capillary columns connected to a 63Ni electron capture detector (GC-ECD). Smaller equipment such as a warring blander, and rotary evaporator were also used during the extraction process. To remove potential phthalate impurities, anhydrous sodium sulfate was refined with acetone and heated for 4 hours at 600°C in a muffle furnace. To record the pesticide chromatogram, a 0.02 µL solution of the standard was injected.

Pesticide extraction from vegetables

Preparation and collection of samples: The sample consists of two hundred fifty grams (250g) of each vegetable, such as bottle gourd (*Lagenaria siceraria*), ridge gourd (*Luffa aegyptiaca*), pointed gourd (*Trichosanthes dioica*), bitter gourd (*Momordica charantia*), and brinjal (*Solanum melongena*) were purchased from the local market of Agra (Sikandra, Khandari, Pratap Pura, and Ram bag), and refrigerated at 5 °C. Each sample was carefully washed with tap water for a few minutes and dried with filter paper. Each sample was cut into small pieces by a grater.

Extraction and clean-up of sample

25 g sample of each vegetable was macerated with 15 g of anhydrous sodium sulfate in a blender. Samples were extracted with 50 ml acetone on a mechanical shaker for 1 h. The acetone extract was collected by vacuum suction and the process was repeated three times for complete extraction. The filtrate was concentrated to near 50 ml using a rotary vacuum evaporator and subjected to liquid-liquid partitioning with n-hexane in a separatory funnel. Then n-hexane layer was collected by passing the sample through the sodium sulfate. The collected layer was evaporated up to 25 ml and purified on a column packed with Florisil and activated carbon (5:1 w/w). The eluted extract was dried, re-dissolved in 5 mL n-hexane, and then injected into GC-ECD for analysis.

Pesticide extraction from fruits

Collection and preparation of samples: The sample contained 250 g of each fruit, consisting of apple (*Malus domestica*), pomegranate (*Punica granatum*), litchi (*Litchi chinensis*), plum (*Prunus domestica*) and papaya (*Carica papaya*) collected from the local markets of Agra (Sikandra, Khandari, Pratap Pura, and Ram bag). Individual samples were cooled at 5°C and analyzed within 24 h from the time of their collection. Each fruit was washed under water for a few minutes and dried with filter paper. After drying, each fruit was cut into small pieces with the help of a grater.

The extraction of apple (Malus pumila), pomegranate (Punica granatum), litchi (Litchi chinensis), plum (Prunus domestica), and papaya (Carica papaya)

50 g fine paste of each fruit sample was taken and mixed with 50 ml acetonitrile (3 x 50 ml) and the extract was filtered by using Buchner funnel. The filtrate was vacuum-concentrated up to 5 mL and transferred to a separatory funnel. Saline solution (2%, w/v,150 ml) was added to it and the extract was exchanged into dichloromethane (3×50 ml) by liquid-liquid partitioning. The extract was passed a layer of sodium sulfate (5g). The extract was evaporated up to 2-5 ml by using a rotatory evaporator. The concentrated extract was dissolved in 10 ml hexane–acetone (9:1v/v).

Purification: The obtained extracts were cleaned using columns filled with silica gel: activated charcoal (5:1 w/w)/silica gel. Each extract was eluted with 50 mL of n-hexane, evaporated up to 5ml, and then subjected to GC-ECD for pesticide analysis.

RESULTS AND DISCUSSION

First, we have determined the retention time and the peak area corresponding to 0.4µg for standards. The chromatogram of the standards showed different peaks corresponding to benzene hexachloride (BHC) isomers, such as alpha-BHC, beta-BHC, gamma-BHC, and delta-BHC, at Rt values 11.883, 13.393, 15.001 and 16.580, respectively. The Rt values at 17.881 18.414, and 18.597 correspond to heptachlor, aldrin, and heptachlor epoxide respectively. The peaks of γ-chlordane and α-chlordane were detected at Rt values 19.527 and 20.088, respectively. Endosulfan I, 4,4'-DDE, and dieldrin's peak were found to be at Rt values 21.287 22.221, and 22.812. Peaks for endrin, 4,4'-DDD, endosulfan II, endrin aldehyde, 4,4'-DDT, and endosulfan sulfate were found at Rt values 23.224, 23.497, 24.598, 24.884, 26.630, and 27.485, respectively. Methoxychlor and endrin ketone exhibited a peak at an Rt value of 32.724, and 34.543, respectively.

Table 1 The table below provides the peak, retention time, area, and pesticide concentration of the OCP standard

Peak	1	2	3	4	5
Pesticide	α-BHC	β-BHC	γ-BHC	δ-BHC	Heptachlor
Ret. Time	11.883	13.393	15.001	16.580	17.881
Area	11,807,373	7,989,110	8,573,327	8,438,721	425,114
Conc. (µg/mL)	200.4	200.0	199.9	200.8	200.8
Peak	6	7	8	9	10
Pesticide	Aldrin	Heptachlor epoxide	γ-chlordane	α-chlordane	Endosulfan - I
Ret. Time	18.414	18.597	19.527	20.088	21.287
Area	7,366,997	227,048	7,960,451	13,851,712	14,657,752
Conc. (µg/mL)	200.4	200.0	200.4	200.0	200.0
Peak	11	12	13	14	15
Pesticide	4,4'-DDE	Dieldrin	Endrin	4,4'-DDD	Endosulfan - II
Ret. Time	22.221	22.812	23.224	23.497	24.598

Area	7437228	6537918	6085168	4873039	6260739
Conc. (µg/mL)	200.0	200.3	200.0	200.0	200.0
Peak	16	17	18	19	20
Pesticide	Endrin aldehyde	4,4'-DDT	Endosulfan sulphate	Methoxychlor	Endrin Ketone
Ret. Time	24.884	26.630	27.485	32.724	34.543
Area	6621085	6851314	3538522	51947	201908
Conc. (µg/mL)	200.0	200.0	200.3	200.4	200.4

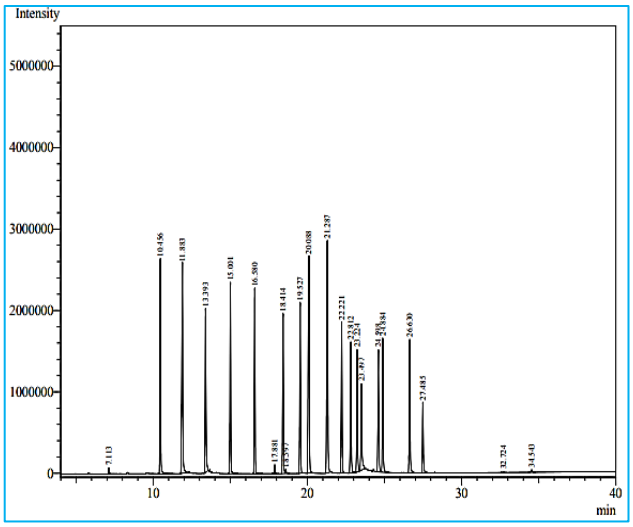

Fig 1 Chromatogram of pesticides standard

In the chromatogram of apple (Fig 2), three peaks at Rt values 11.878, 16.545, and 18.509, were very close to the Rt values of α-BHC, δ-BHC, and heptachlor epoxide indicating the presence of these pesticides in the sample of apple. However, pomegranate (Fig 3) exhibited several peaks among them the peaks at Rt values 16.550, 18.507, and 22.880, were close to the Rt values of δ-BHC, heptachlor epoxide, and dieldrin, respectively [22-25]. Six peaks at Rt values 16.557, 18.507, 20.067, 21.279, 22.807, and 34.573, were very near to the Rt values δ-BHC, heptachlor epoxide, α-chlordane, endosulfan – I, dieldrin and endrin ketone respectively in the chromatogram of litchi (Fig 4). Three peaks at the Rt value of 17.823, 18.505, and 34.571, were very close to the Rt values of Heptachlor, Heptachlor epoxide, and Endrin ketone respectively in the chromatogram of plum (Fig 5). Two peaks were found very close in the chromatogram (Fig 6) of papaya at Rt values of 16.544, and 18.500, to the Rt values of δ-BHC, and heptachlor epoxide respectively.

The chromatograms of the bottle gourd (Fig 7) and ridge gourd (Fig 8) exhibited several peaks among them peaks at Rt value 18.509 and 18.507 respectively indicating the presence of heptachlor epoxide in both samples. Two peaks at Rt values 16.542 and 18.518, were found very near to the Rt values δ-BHC and heptachlor epoxide respectively in the chromatogram (Fig 9) of the pointed gourd [26-27]. Two peaks at the Rt values 16.549, and 18.518, were very close to the Rt values of δ – BHC, and Heptachlor epoxide, respectively in the chromatogram of bitter gourd (Fig 10).

In the chromatograms of the brinjal (Fig 11) peak at Rt values 18.522, was very close to heptachlor epoxide Rt [28]. The detected pesticides and their concentrations are shown below in (Table 2).

Table 2 Detected pesticides and concentration of pesticides in fruit and vegetable samples by using GC-ECD

Name of the samples	Detected pesticides	Ret. Time	Area	Concentration of pesticides (µg/µL)
Apple	α- BHC	11.878	4888	0.00008
	δ - BHC	16.545	13115	0.00031
	Heptachlor epoxide	18.509	137620	0.12122
Pomegranate	δ - BHC	16.550	11589	0.00027
	Heptachlor epoxide	18.507	1792351	0.15788
	Dieldrin	22.880	7103	0.00021
Litchi	δ – BHC	16.557	4604	0.00010
	Heptachlor epoxide	18.507	803865	0.70810
	α - chlordane	20.067	3231	0.00004
	Endosulfan I	21.279	2508	0.00003
	Dieldrin	22.807	3156	0.00009
	Endrin ketone	34.573	11434	0.01132
Plum	Heptachlor	17.823	18756	0.00882
	Heptachlor epoxide	18.505	1756176	1.54696
	Endrin ketone	34.571	13011	0.01288
Papaya	δ – BHC	16.544	4863	0.00011
	Heptachlor epoxide	18.500	1632201	1.43775
Bottle Gourd	Heptachlor epoxide	18.509	154216	0.13584
Ridge Gourd	Heptachlor epoxide	18.507	120392	0.10604
Pointed Gord	δ - BHC	16.542	11844	0.00028
	Heptachlor epoxide	18.518	15698	0.01382
Bitter Gourd	δ – BHC	16.549	7608	0.00018
	Heptachlor epoxide	18.518	342825	0.30198
Brinjal	Heptachlor epoxide	18.522	520502	0.45849

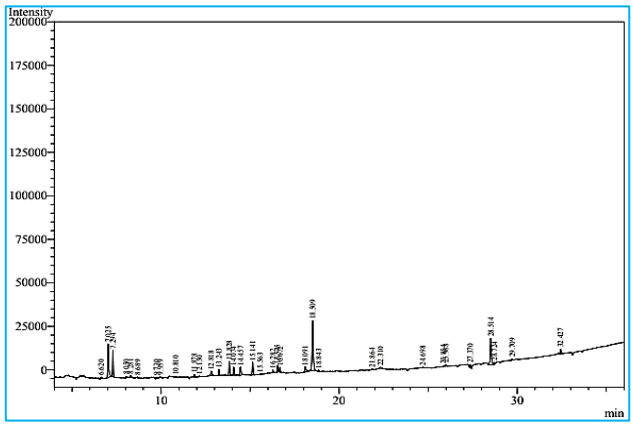

Fig 2 Chromatogram of pesticide present in apple

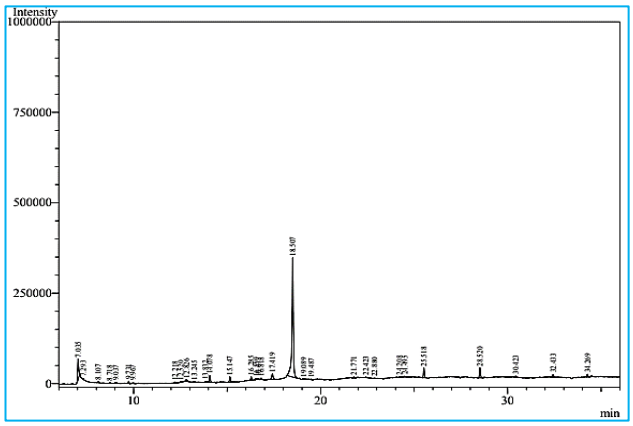

Fig 3 Chromatogram of pesticide in pomegranate

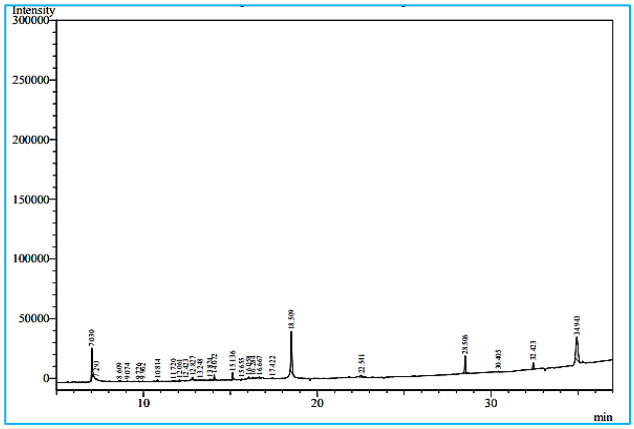

Fig 4 Chromatogram of pesticide present in litchi

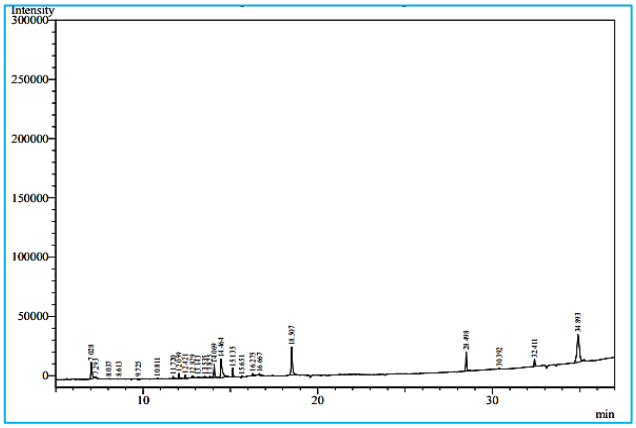

Fig 5 Chromatogram of pesticide in plum

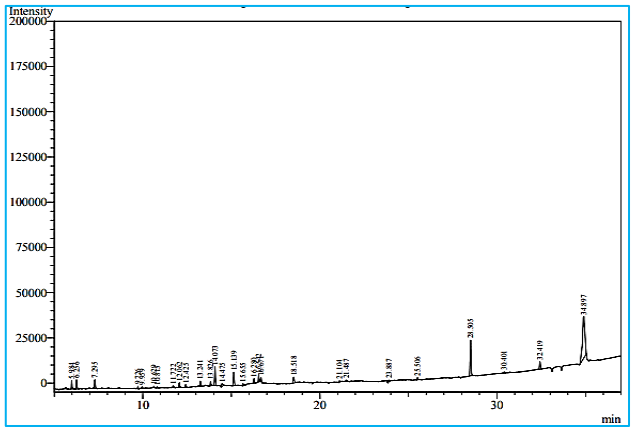

Fig 6 Chromatogram of pesticide present in papaya

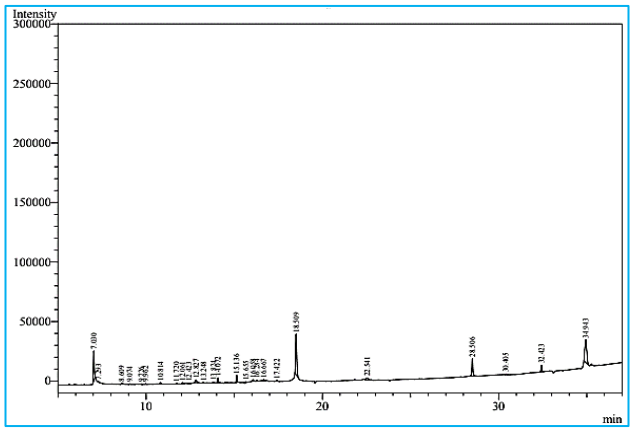

Fig 7 Chromatogram of pesticide present in bottle gourd

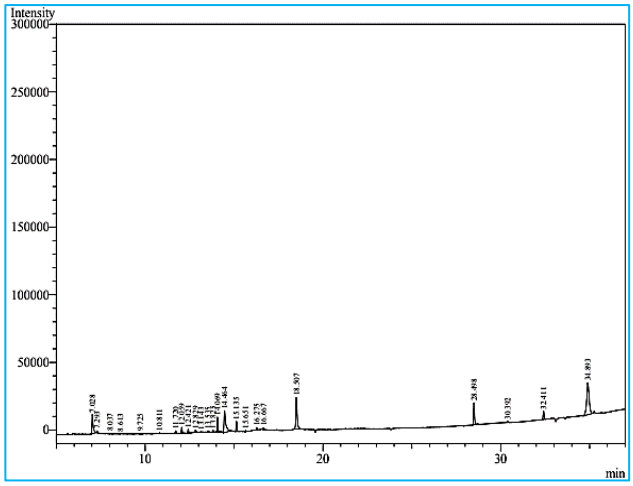

Fig 8 Chromatogram of pesticide present in ridge gourd

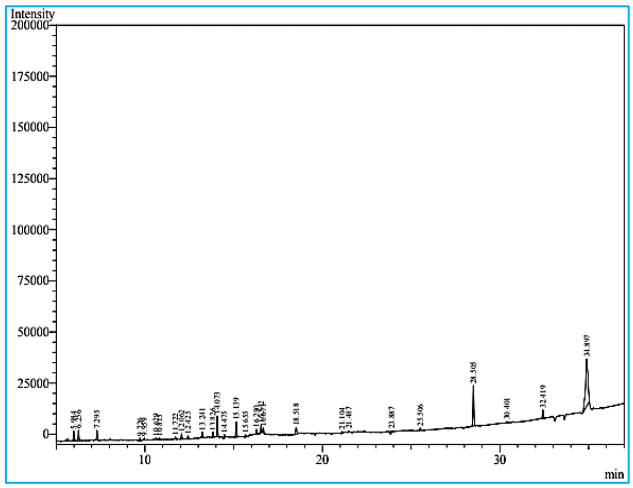

Fig 9 Chromatogram of pesticide present in the pointed gourd

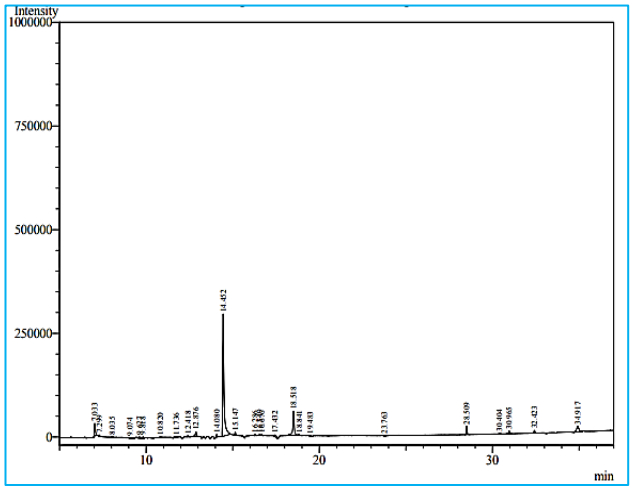

Fig 10 Chromatogram of pesticide present in bitter gourd

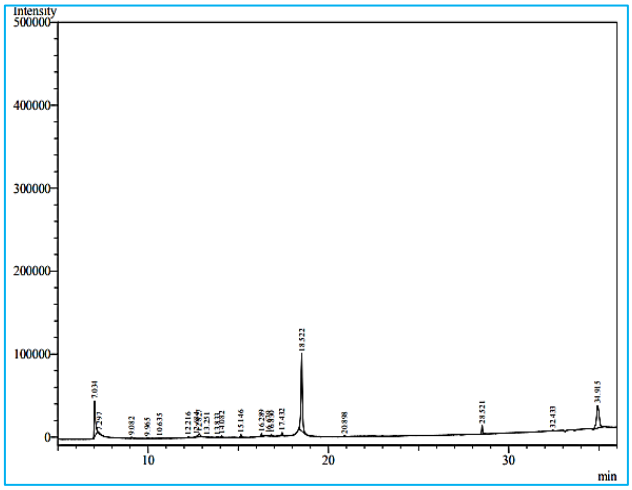

Fig 11 Chromatogram of pesticide present in the brinjal

CONCLUSION

Rapid monitoring of pesticide residues in fruits and vegetables is important and necessary to reduce health risks; Therefore, a simple, effective, and rapid method was developed to monitor organochlorine pesticides using gas chromatography equipped with an electron capture detector. The obtained results indicated that both the types of fruits and vegetables samples viz. Apple, pomegranate, litchi, plum, papaya and bottle gourd, ridge gourd, pointed gourd, bitter gourd, and brinjal were contaminated with heptachlor epoxide in maximum concentration, while α- BHC, δ-BHC, dieldrin, endosulfan I, α - chlordane, heptachlor, and endrin ketone in minimum concentration.

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