Chapter 7

Gas Chromatography-Mass Spectrometry Multi-residue Screening of Chlorinated Pesticides in/on Brinjal

CONTENTS

1.   Introduction

Pesticides are the integral part of intensive agriculture. Brinjal (Solanum melongena L.) is one of the common and popular vegetable grown in India with the production of 31,24,487 tones per annum[1]. Several insecticides are recommended for the control of brinjal shoot and fruit borer and other sucking pests. The majority of the chlorinated pesticides being non-biodegradable leave residues in various food commodities. Residues of these insecticides in/on vegetables should be below maximum residues limits (MRLs), fixed by various organizations like Food Agriculture Organization (FAO), United States Environmental Protection Agency (US EPA) and European Union (EU)[2]. The safety concerns for the environment and public health have spawned more stringent analytical testing requirements, for the determination of the pesticide residues in food at a very low levels as well as the confirmation of their identity.

A number of multi-residue procedures have been published for the determinations of chlorinated pesticides in various vegetables and fruits using a gas chromatograph with electron capture detector (GC-ECD)[3,4]. But the GC-ECD data does not provide sufficient information for confirmation of the compound. Therefore many articles reported the use of a GC-MS system[5-7] along with GC-ECD for the confirmation of the residues, as the mass selective detector (MSD), in Scan mode (total-ion-monitoring mode) is very useful for identification of a compound with mass spectral information. But it needs two different instrumental analyses of sample extracts for quantitation and identification. Therefore numerous GC-MS SIM mode methods[8-11] have been developed, which were suitable for both identification and quantitation of lower level multi-residues of pesticides due to increases sensitivity of target analyte through the selected detection of ions.

The majority of the methods published have used various techniques viz., solid phase extraction (SPE)[5,6,12], solid phase micro extraction (SPME)[13,14], matrix solid phase dispersion (MSPD)[15], dispersive solid phase extraction[16], ultrasonic extraction[17] or gel

permeation chromatography[18] to remove matrix interferences and to obtained purified extracts. These extraction and clean-up procedures are expensive, tedious and time-consuming.

The objective of the present work was to develop a simple, rapid and efficient extraction technique with an easy analytical procedure for multi-residue detection of thirteen selected chlorinated pesticides in brinjal. Attempts were made to extract pesticide residues from brinjal samples with specific combination of solvents using ultrasonic extraction followed by centrifugation without any clean-up or derivitization step. The extracts were analysed by a gas chromatograph coupled with mass spectrometer (GC-MS, SIM) using selected qualifier ions for each of the pesticides. The method provided simultaneous identification and quantitation of thirteen chlorinated pesticides and showed good sensitivity and selectivity as compared to other conventional methods.

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 Mass Selective Detector (GC/MS)	6890/5973	Hewlett Packard, USA
3	GC Column [30 m x 0.25 mm (i.d.) x 0.25 μm film thickness]	HP-5 MS	Hewlett Packard, USA
4	Automatic Injector	6890 Series	Hewlett Packard, USA
5	Orbital Shaker (Rotary Shaker)	T_1-641/642	Tempo, India
6	Sonicator (Ultrasonic Bath)	UCH 500 W	Laboratory Instrument, India
7	Refrigerated Centrifuge	Rota 6R – V/F_M	Plasto Craft Industries Pvt. Ltd., India
8	Rotary Vacuum Evaporator	02257 Superfit	Plasto Craft Industries Pvt. Ltd., India
9	Mixer Blender	Sumeet	-

2.2 Solvents and Chemicals

Sr. No.	Solvents/Reagents	Grade	Supplier
1	Acetone	ExcelaR	Qualigens, India
2	Dichloromethane (DCM)	ExcelaR	Qualigens, India
3	n-hexane	ExcelaR	Qualigens, India
4	Potassium Ortho Phosphate	Laboratory Reagent	Glaxo, India
5	Anhydrous Sodium Sulphate	Laboratory Reagent	Glaxo, India

2.3 Standards and Samples

The certified reference standards of HCH, lindane, endosulfan-I, endosulfan-II, heptachlor, alachlor, aldrin, m-chlorothalonil, butachlor, dieldrin, p,p'-DDT, endrin and methoxychlor, were procured from Chem. Service (West Chester, PA USA).

Samples of brinjals were drawn from the market. The brinjal samples, which were found free from pesticides, were used as blank matrix.

2.4 Standard Mixture of Pesticides

The standard stock solution of each pesticide was prepared at 1 mg/ml concentration, by weighing 10 mg (approx.) reference standard of each pesticide into separate 10 mL volumetric flasks, and volume was made upto the mark with n-hexane. A stock standard mixture was prepared by transferring the 1 mL standard stock solution of each pesticide into a 100 mL volumetric flask and the volume was made upto the mark with n-hexane. The resultant stock mixture contains 10 ppm (μg/ml), approx. of each pesticide, which was analyzed by GC-MS after suitable dilution. All standard and stock solutions were stored in refrigerator at 4 °C.

2.5 Sampling and Spiking Procedure

1 kg of brinjal samples were chopped into small pieces, blended at high speed in a mixer to pulverize and homogenize and 100 g representative sample was drawn and spiked in duplicate at 20 μg /kg level with standard stock mixture of pesticides. Spiked samples were thoroughly mixed and allowed to stand overnight at room temperature in a closed fume hood to avoid contamination.

2.6 Extraction Procedure

10 g representative spiked sample was transferred into a 250 mL reagent bottle, mixed with 10 mL solution of 10% potassium ortho-phosphate and 100 mL solvent mixture of acetone-dichloromethane-hexane (40:30:30, v/v/v). The solution was shaken for 15 min in an Orbital Shaker and then sonicated for 15 min in a Sonicator (Ultrasonic Bath) and finally centrifuged at 4000 rpm for 5 min in a Refrigerated Centrifuge. The extraction procedure of sonication amd centrifugation was repeated thrice with 50, 50 and 25 mL solvent combination. The organic phase was collected through a bed of anhydrous sodium sulphate and combined phase was concentrated to dryness using a Rotary Vacuum Evaporator at <40 °C. The residue was dissolved in 5 mL n-hexane and analyzed by GC-MS system.

2.7 Gas Chromatograph-Mass Spectrometer Conditions

The standard and sample solutions were injected onto GC/MS using following parameters:

Instrument	:	GC/MS (Hewlett Packard-6890/5973) with HP ChemStation Data System
Column	:	HP-5, MS; [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness] (5% phenyl and 95% methyl siloxane stationary phase)
Column oven temperature:		110 °C to 250 °C @ 8 °C/min (hold for 2.5 min); 250 °C to 280 °C @ 10 °C/min (hold for 4.0 min)
Injector port temperature	:	260 °C
Transfer line temperature	:	280 °C
Injection mode	:	Splitless (Purge flow : 50 mL/min.)
Injection volume	:	1 µL
Carrier gas	:	Helium
Carrier gas flow rate	:	1.0 mL/min
Detector	:	Mass selective detector (MSD)
Source temperature	:	230 °C
Quadrupole temperature	:	150 °C
Electron Impact	:	70 eV
Ionization mode	:	Scan mode

Mass range : 50 to 500 m/z

Filament (solvent) delay : 4.0 min.

Quadrupole temp : 150 °C

Ionization mode : SIM mode

Groups & selected ions : Refer **Table 1**

Table 1: GC-MS SIM parameters with selected qualifier ions for various pesticides.

| Group	Pesticides	Start Time	Cycles/sec.	Selected Qualifier Ions				Dwell Time (sec/min.)
1	α-HCH	4.00	2.15	111	181	198	219	100
2	γ-HCH (Lindane)	9.42	1.72	111	147	181	219	100
3	m-Chlorothalonil	10.12	2.86	264	266	268	-	100
4	Heptachlor	11.21	1.44	237	270	272	274	100
5	Alachlor	11.21	1.44	160	188	237	-	100
6	Aldrin	11.96	2.15	261	263	265	293	50
7	Endosulfan-I	13.75	2 15	195	237	239	249	100
8	Butachlor	13.94	2.86	176	160	273	-	50
9	Diendrin	14.35	1.72	79	263	277	279	50
10	Endosulfan-II	15.06	2.15	195	237	239	241	100
11	p,p'-DDT	15.81	2.86	165	199	235	-	100
12	Endrin	17.08	1.72	181	250	315	319	50
13	Methoxychlor	17.47	2.86	212	227	228	-	100

The most abundant and characteristic qualifier ions were selected for each pesticide in the mixture for identification and the quantitation (**Table 1**). The pesticides in sample were subsequently identified by comparing the retention times and ratio of qualifier ions with standard mixture.

3.   Results and Discussion

3.1   Validation

The GC/MS analytical method was capable of simultaneous analysis of thirteen pesticides within 20 min. Some typical GC/MS chromatograms of pesticides mixture at 0.5 ppm, 0.1 ppm, 0.05 ppm and 0.05, 0.005 and 0.001 ppm concentrations were presented at scan mode (total ion monitoring) and SIM mode (selected ion monitoring) of analysis, respectively **(Fig. 1 & 2)**. The total ion chromatograms of scan mode analysis showed higher baseline noise, which was minimum in SIM mode analysis due to monitoring of selected ions. Therefore, SIM chromatograms had much-improved signal to noise ratio (S/N) and about 100 times more sensitivity than scan mode analysis. The changes in baseline during SIM mode analysis were due to change of groups with time **(Table 1)**.

The limit of detection (LOD) was considered at the lowest concentration that produces a response three times of the average baseline noise (S/N=3). The limits of detection of all pesticides in Scan and SIM mode were determined using both standard solutions and spiked samples **(Table 2)**.
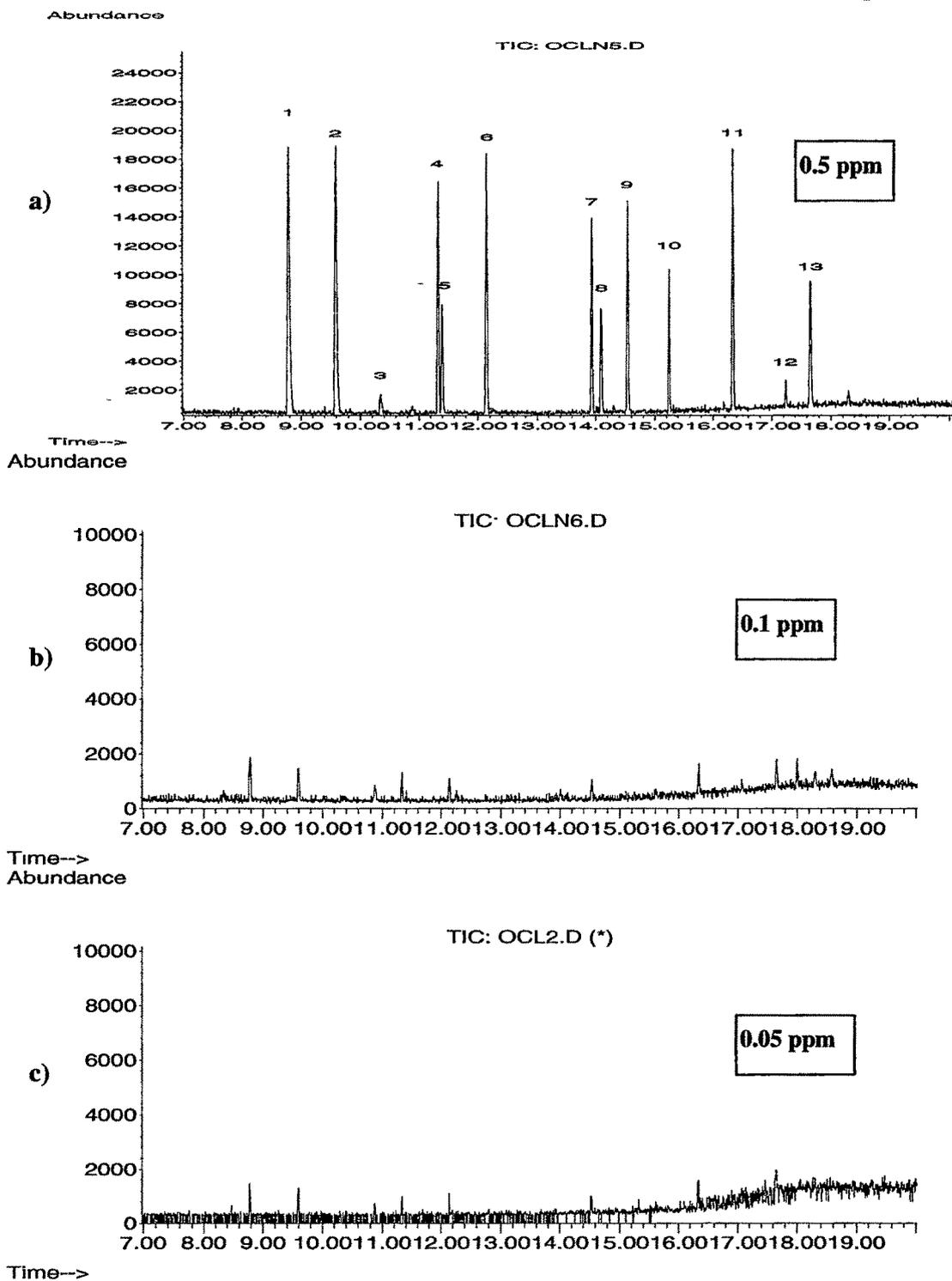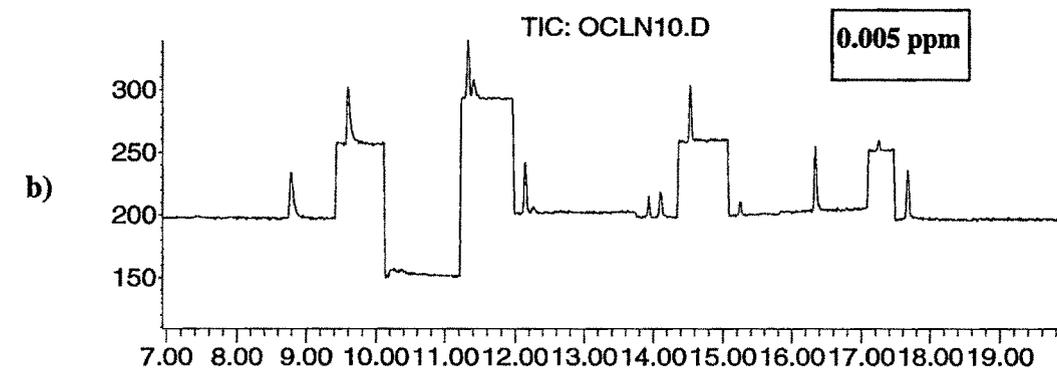
Fig. 1: GC/MS-Scan mode chromatograms for std. mixture of chlorinated pesticides a) 0.5 ppm, b) 0.1 ppm, c) 0.05 ppm.

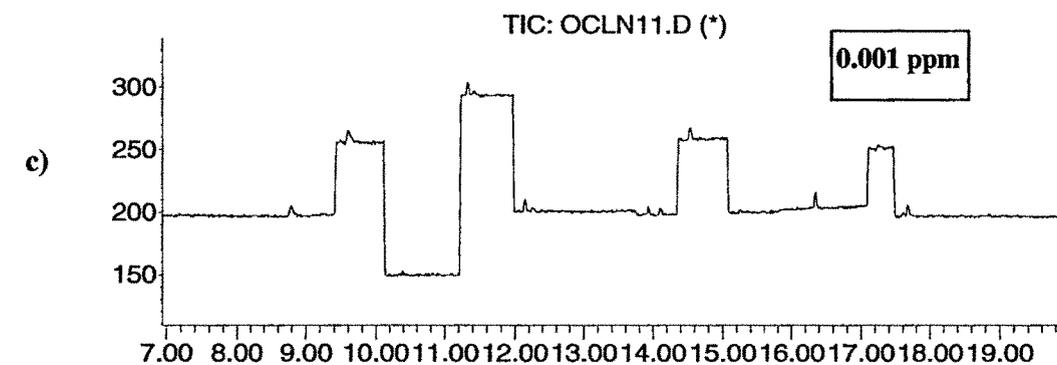[Refer Table 1, page 141 for peak names]
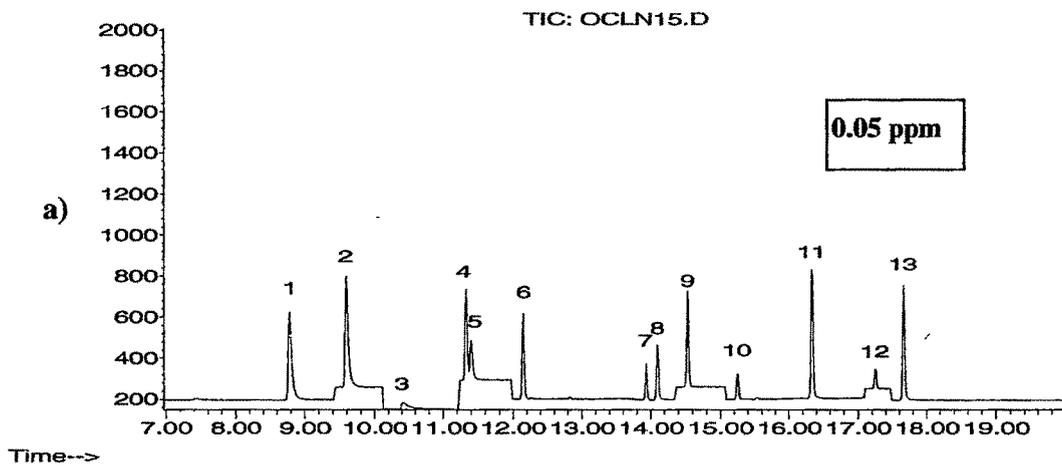
Abundance

Fig. 2:   GC/MS-SIM mode chromatograms for std. mixture of chlorinated
pesticides a) 0.05 ppm, b) 0.005 ppm, c) 0.001 ppm.

[Refer Table 1, page 141 for peak names]

The LOD of instrument with Scan mode using standard solutions of pesticides ranged from 0.05 to 0.2 ppm, while with SIM mode the maximum sensitivity varied from 0.0005 to 0.001 ppm. The limit of detection of the method by analyzing spiked samples in SIM mode, ranged from 0.001 to 0.005 mg/kg. The method shows better sensitivity and selectivity in comparison to other conventional methods viz., GC-MS and GLC-ECD methods.

Linear responses were checked for all the pesticides over a wide concentration range (**Fig. 3**). With Scan mode, the correlation coefficient (r) of pesticides at concentration from 0.1 to 1 ppm varied from 0.9982 to 0.9999, while with SIM mode at concentration from 0.001 to 0.1 ppm, the correlation coefficients ranged between 0.9982 to 0.9999.
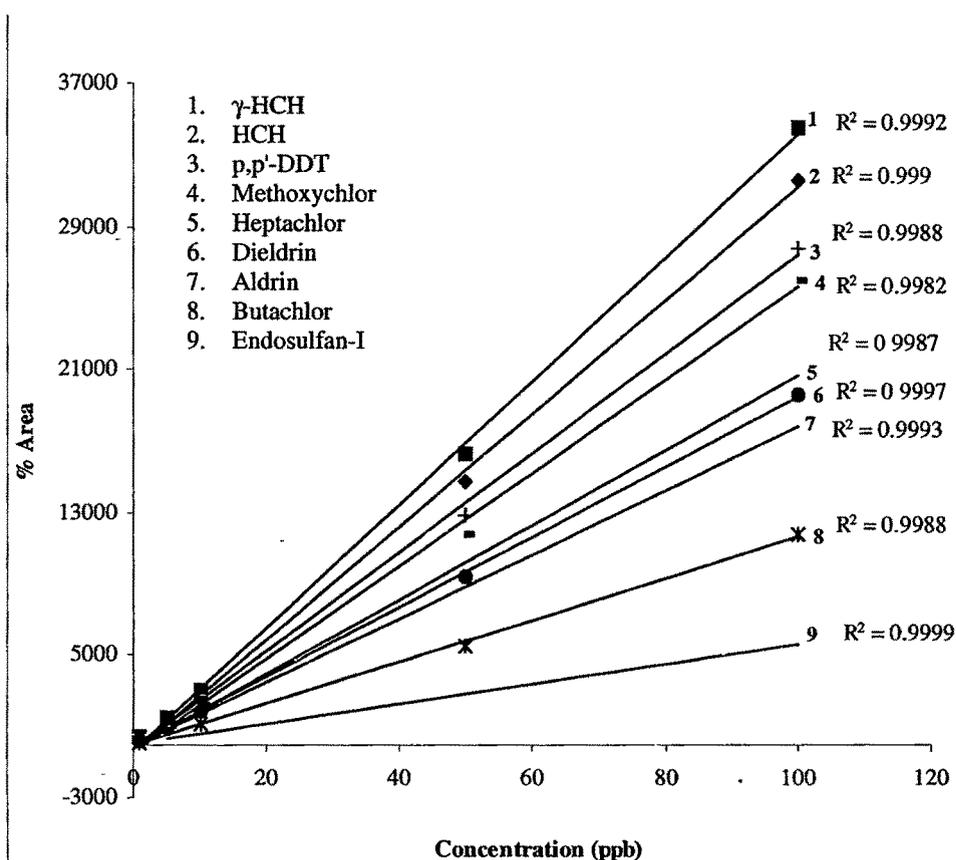
Fig. 3: Calibration curves of some chlorinated pesticides (0.001 to 0.1 ppm) with GC-MS SIM mode analysis.

The intra-assay relative standard deviation (% RSD) for the pesticides using five repeated injections of pesticides mixture, were 1.1 to 2.6% in scan mode (at 1.0 µg/ml level), and 1.0 to 4.5% in SIM mode analysis (at 0.005 µg/ml concentration level) (**Table 2**).

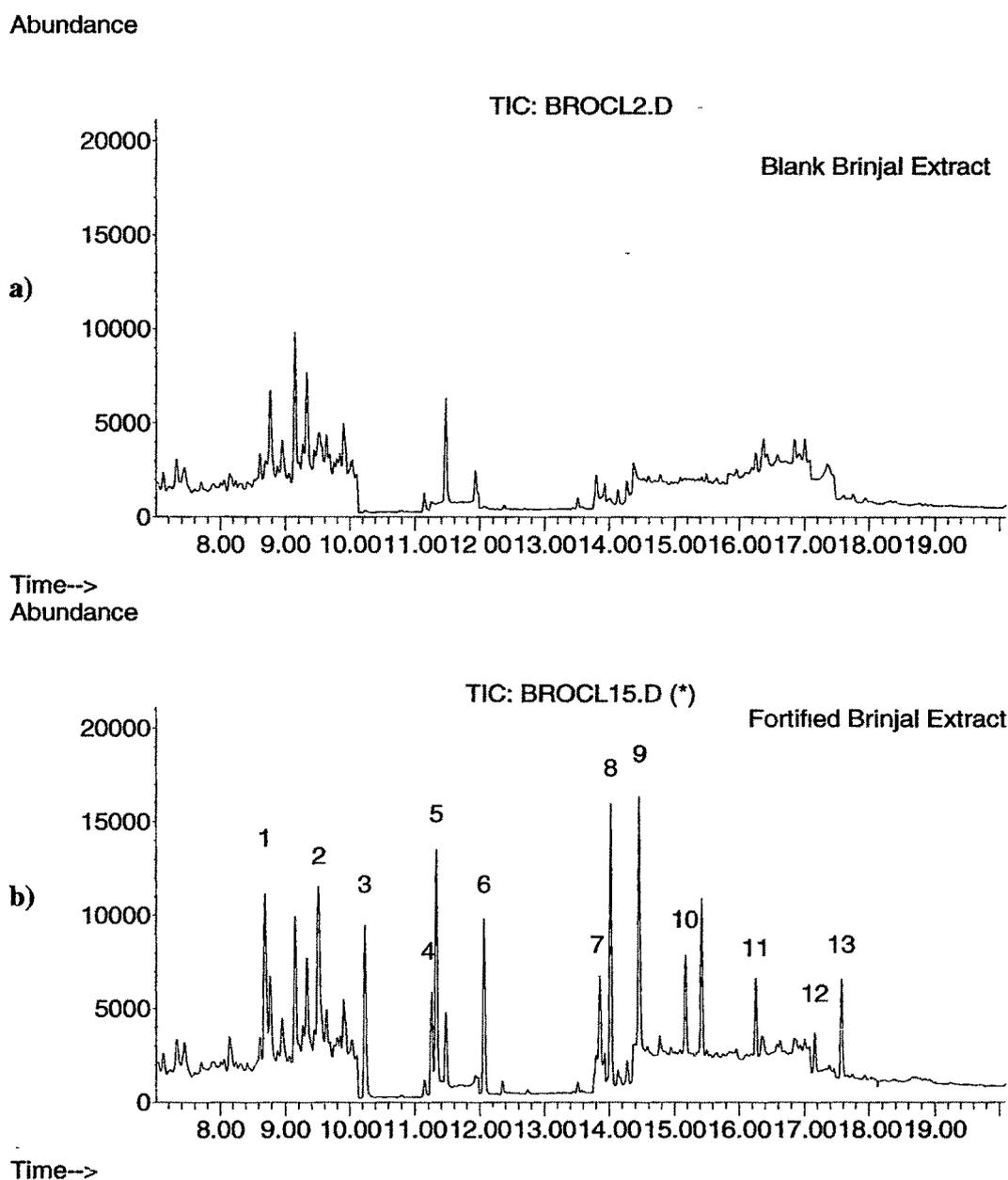**Table 2:   Validation results of GC-MS analytical method [Scan and SIM mode].**

| Sr. No. | Compounds | R.T. | % RSD (Scan) | % RSD (SIM) | Limit of Detection | | | Average Recovery (Brinjal) (%) |
| | | | | | Instrument | | Method | |
					Scan (ppm)	SIM (ppm)	SIM (mg/kg)	
1	α-HCH	8.77	1.38	1.14	0.050	0.0005	0.001	87.87
2	γ-HCH (Lindane)	9.58	1.38	2.20	0.050	0.0005	0.001	111.52
3	m-Chlorothalonil	10.37	2.49	4.51	0.200	0.0005	0.001	104.12
4	Heptachlor	11.33	1.85	1 80	0.050	0.0005	0.005	74.00
5	Alachlor	11.40	2.48	1.01	0.100	0.0010	0.001	78.81
6	Aldrin	12.15	1.52	1.99	0.050	0.0005	0.001	76.10
7	Endosulfan-I	13.93	1.11	3.52	0.200	0.0005	0.005	104.81
8	Butachlor	14.10	1.44	2.49	0.200	0.0005	0.001	84.26
9	Diendrin	14.54	1.81	1.70	0.050	0.0005	0.001	100.40
10	Endosulfan-II	15.25	2.01	3.34	0.200	0.0010	0.005	87.59
11	p,p'-DDT	16.34	1.80	2.54	0.050	0.0005	0.005	90.88
12	Endrin	17.24	2.62	1.82	0.200	0.0010	0.005	73.17
13	Methoxychlor	17.66	2.64	2.69	0.050	0.0005	0.001	81.47

3.2   Matrix Effect

The typical chromatograms of GC/MS-SIM mode analysis of a blank brinjal extract and a brinjal sample, spiked with a pesticides mixture at 20 µg/kg level (**Fig. 4**) showed low background signals and negligible interference of matrix with the analytes. Therefore the extract needed no further clean-up procedure. The average recoveries of pesticides from various spiked brinjal samples at 20 µg/kg level varied between 73 to 112% with RSD less than 15%.

During the pesticides extraction from brinjal samples, the critical problem was the selection of optimum solvent combination for satisfactory recoveries of all thirteen pesticides. Several combinations of different solvents were used for efficient solvent combination. In acetone-dichloromethane-hexane mixture, acetone portion was fixed for best penetration in the vegetables. The mixture of acetone and dichloromethane was sufficiently polar to extract all pesticides, where as n-hexane minimizes the extraction of polar co-extractives. The combinations of more polar solvents (methanol, acetonitrile) were also tried, but were not suitable for pesticides and co-extractives partitioning. The solvent combination of acetone-dichloromethane-hexane (40:30:30, v/v/v) was most suitable and gave satisfactory recoveries of all thirteen pesticides with low background interference.

Despite the removal of the clean-up procedure (column or gel chromatography, SPE or SPME) or any derivitization before GC-MS analysis, the extract in the present technique was relatively clean without sacrificing the sensitivity of the method (**Fig. 4**). The routine applicability of the method was verified on brinjal samples and found that the pesticides were either absent or at very low levels.

Abundance

TIC: BROCL2.D

Blank Brinjal Extract

a)

Time-->
Abundance

TIC: BROCL15.D (*)

Fortified Brinjal Extract

b)

Time-->

Fig. 4: **a) Blank brinjal extract, b) Fortified brinjal extract at 20 µg/kg (0.02 ppm) level.**

[Refer Table 1, page 141 for peak names]

4. Conclusion

A simple, fast, efficient and economic GC-MS, SIM mode method was developed for simultaneous identification and quantitation of multi-residues of thirteen chlorinated pesticides in/on brinjal samples, with a rapid extraction procedure using a solvent mixture of acetone-dichloromethane-hexane. The resolution of all the pesticides was excellent, within a run time of approximately 20 min. The limit of detection (LOD) of instrument varied from 0.0005 to 0.001 ppm (ng/μL) using standard solutions, while LOD of method varied from 0.001 to 0.005 mg/kg with spiked samples. The relative standard deviation was less than 5% for all pesticides and the correlation coefficients (r) over concentration range of 0.001 to 0.1 ppm, varied from 0.9982 to 0.9999. The recoveries of pesticides from spiked brinjal samples at 20 μg/kg level, varied from 73 to 112%. Therefore, the proposed method is very useful for routine residue analysis (identification and quantification) of chlorinated pesticides from brinjal samples.

5. References

1. *Annual Report*, Assam Agriculture University, All India Co-ordinated Vegetable Improvement Project (ICAR), Jorhat Center, AAU (1991).

2. European Union (EU) Community Directive 93/58/EEC, *Off. J. Eu. Commun. L.*, European Community Brussels Belgium, 211/6, (1993).

3. Pang, G. F., Cao, Y. Z., Fan, C. L., Zhang, J. J. and Li, X. M., *J AOAC Int,* **82**, 186 (1999).

4. Fernández-Alba, A. R., Valverde, A., Agüera, A., Contreras, M., *J. Chromatogr. A* **686**, 263 (1994).

5. Columé, A., Cárdenas, S., Gallego, M., Valcárcel, M., *J. Agric. Food Chem.* **49**, 1109 (2001).

6. Columé, A., Cárdenas, S., Gallego, M., Valcárcel, M., *J. Chromatogr. A* **882**, 193 (2000).

7. Gelsomino, A., Petrovicová, B., Tiburtini, S., Magnani, E., Felici, M., *J. Chromatogr. A*, **782**, 105 (1997).

8. Lacassie, E., Dreyfuss, M. F., Daguet, J. L., Vignaud, M., Marquet, P., Lachâtre, G., *J. Chromatogr. A* **805**, 319 (1998).

9. Reyzer, M. L. and Brodbelt, J. S., *Anal. Chim. Acta,* **436**, 11 (2001).

10. Sojo, L. E., Brocke, A., Fillion, J., Price, S. M., *J. Chromatogr. A* **788**, 141 (1997).

11. Soleas, G. J., Yan, J., Hom, K. and Goldberg, D. M., *J. Chromatogr. A,* **882**, 205 (2000).

12. Doong, R., Lee, C., *Analyst* **124**, 1287 (1999).

13. Donga, C., Zeng, Z., Lia, X., *Talanta* **66**, 721 (2005).

14. Yao, Z., Jiang, G., Liu, J. and Cheng, W., *Talanta* **55**, 807 (2001).

15. Chu, X. G., Hu, X. Z., Yao, H. Y., *J. Chromatogr. A* **1063**, 201 (2005).

16. Anastassiades, M., Lehotay, S. J., Stajnbaher, D. and Schenck, F. J., *J. AOAC Int.* **86**, 412 (2003).

17. Lacassie, E., Dreyfuss, M. F., Daguet, J. L., Vignaud, M., Marquet, P., Lachâtre, G., *J. Chromatogr. A* **805**, 319 (1998).

18. Wan, H. B., Wong, M. K., Lim, P. Y., Mok, C. Y., *J. Chromatogr.* **662**, 147 (1994).