Chapter 3

Development of Methods for Impurity Profile Analysis of Technical Grade Pesticides by Gas Chromatograph Coupled with Mass Spectrometer (GC-MS)

CONTENTS

1. Introduction

The chemical composition of a technical grade pesticide is a matter of great concern of regulatory authorities. Technical pesticides, besides active ingredients, also contain complex mixture of minor impurities viz., isomers, raw materials, intermediates, by-products, degradation products or metabolites. These impurities may arise during synthesis due to process variables, side reactions and impurities of raw materials or during storage. Even at the relatively low concentration in technical grade pesticide, impurities may be equally or more toxic than the active ingredient. Various regulatory agencies (EPA[1], PSD[2]) and UN organizations (FAO[3], WHO[4]) have prescribed several guidelines and specifications with limits for active ingredient contents and associated impurities in technical grade materials. Therefore, the identification and quantification of each of the relevant impurities is very important. Some of the impurities present in various technical grade pesticides and their analytical methods have been reported by FAO, WHO as well as in published literature[5-9]. The nature and amount of impurities present in technical grade pesticides may vary based on manufacturing process. Therefore, appropriate analytical methods for the detection / identification and quantitation of low-level impurities is vital.

To develop a suitable analytical method for simultaneous analysis of active ingredient and associated impurities is tedious work. The method should separate all the impurities from active ingredient. The appropriate identification and quantification of each impurity below the permissible levels is another challenge for the analytical chemists. There are various specific detectors viz., Electron Capture Detector (ECD), Nitrogen Phosphorous Detector (NPD) etc., which are very sensitive but not suitable for impurity profile analysis due to their selective detection. The method should universally detect all types of impurities present along with active ingredient; otherwise a combination of various chromatographic and spectroscopic methods needs to be employed for the analysis of

probable impurities in technical products. High-resolution GC/MS is a most suitable instrument for impurity profile analysis of heat stable technical grade pesticides. Since the gas chromatograph can efficiently separate the impurities and isomers, while mass spectrometer, a universal detector can identify and quantify at ppb levels (**Fig. 1**). The identification is based on elution pattern and retention time in GC chromatogram and mass and fragmentation pattern in the mass spectrum of each component.

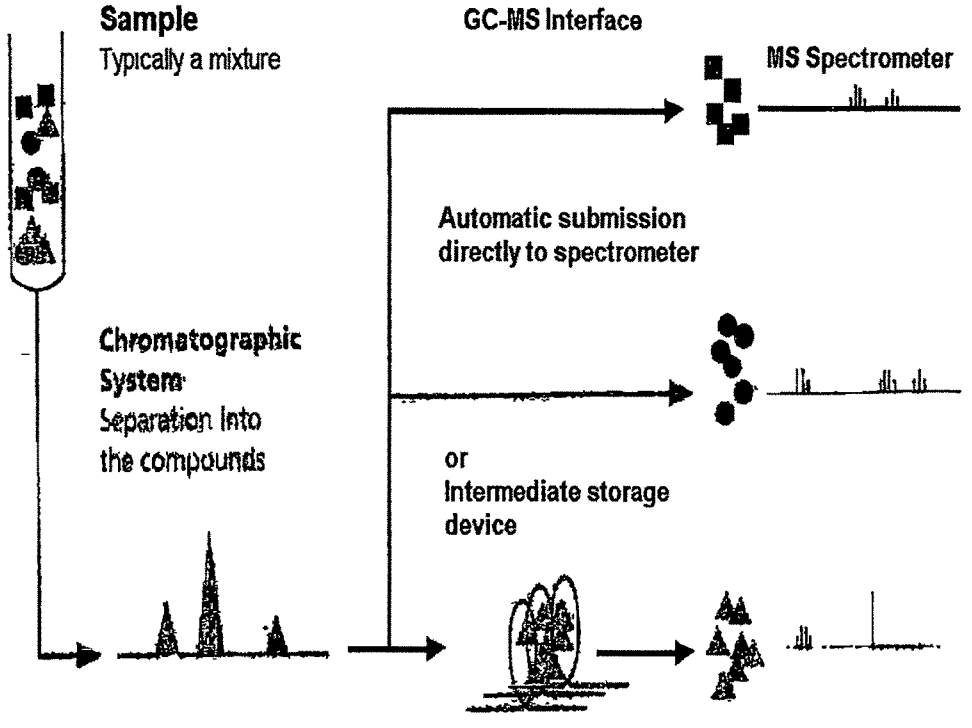


Fig. 1: Functional process of GC/MS for simultaneous separation (GC) and identification (MS) of each component of a complex mixture.

In the present work an attempt has been made to develop the suitable GC/MS analytical methods for impurity profile analysis of eight selected technical pesticides viz., tebuconazole, acephate, chlorpyrifos, metalaxyl, ethofumesate, HCH, chlorothalonil, metribuzin. The development of analytical method for impurity profile analysis of technical grade pesticides consists of following steps:

1. Development of a suitable GC/MS analytical method to scan the technical pesticide with satisfactory separation of all impurities and active ingredient.
2. Identification of possible impurities that may occur by studying the synthesis processes during manufacturing, raw materials and intermediates.
3. Elucidation the structure of all impurities based on their mass and fragmentation pattern.
4. To procure the standards for all the impurities and active ingredient through purchase, synthesis or by isolation from samples.
5. Simultaneous analysis of all the impurities and active ingredient by injecting the standard synthetic mixture of active ingredient and impurities as well as technical sample onto GC/MS.
6. Validation of GC/MS method for each impurity and active ingredient, separately covering the aspects viz., (i) characterization (ii) specificity (iii) linear dynamic range (LDR), (iv) limit of detection (LOD), (v) limit of quantitation (LOQ), (vi) precision (% RSD) [repeatability /reproducibility] and (vii) accuracy (% Recovery).

After validating the GC/MS methods for simultaneous analysis of active ingredient and associated impurities of eight technical grade pesticides, the chemical composition of each technical grade pesticide was determined by quantitative analysis of all associated impurities and active ingredient. The % contents (w/w) of active ingredient and all impurities were calculated by comparing the peak area of each component of sample with standard mixture of known concentration.

2. Methodology for A Typical Impurity Profile Analysis (Tebuconazole)

Common Name : Tebuconazole Technical

C A Name : (±)-α-[2-(4-chlorophenyl)ethyl]- α-(1,1-dimethylethyl)-1H-1,2,4-= triazole-1-ethanol

CAS No. : [107534-96-3]

Molecular Formula : $C_{16}H_{22}ClN_3O$

Molecular Weight : 307.8

Melting Point : 105 °C

Description : Colourless powder

Solubility : Dichloromethane >200 g/L, Isopropanol 50 - 100 g/L at 20 °C.

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	Turbo Pump	G109980024	Hewlett Packard, USA
4	Auto Sampler (6890 series injector)	G1512A	Hewlett Packard, USA
5	GC Column [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]	HP-5 MS	Hewlett Packard, USA
6	Data System	ChemStation	Hewlett Packard, USA

2.2 Solvents and Chemicals

Sr. No.	Solvents/Reagents	Grade	Supplier
1.	Dichloromethane	ExcelaR	Qualigens, India

2.3 Reference Standards

Sr. No.	Pesticides	Purity (% w/w)	Source
1.	Tebuconazole	99.0	Sigma-Aldrich, USA
2.	Impurity-1	98.6	Jai Research Foundation, India
3.	Impurity-2	97.8	Jai Research Foundation, India
4.	Impurity-3	98.0	Jai Research Foundation, India
5.	Impurity-4	98.5	Jai Research Foundation, India

Note: The impurities names have been coded, because it is considered to be commercially confidential information.

2.4 Preparation of Standard Mixture Solution

The standard stock solutions of impurities were prepared by accurately weighing a known quantity (10 mg approx.) of each of the tebuconazole associated impurities (viz., Imp-1, Imp-2, Imp-3 and Imp-4) into separate volumetric flasks of 10 mL capacity, contents were dissolved in 5 mL dichloromethane and volume was made upto the mark with dichloromethane. The standard mixture of tebuconazole and associated impurities was prepared by accurately weighing a known quantity of tebuconazole reference standard (10 mg approx.) into a volumetric flask of 10 mL capacity and transferring 0.1 mL each of above prepared standard stock solutions of impurities into the same volumetric flask and volume was made upto the mark with dichloromethane.

2.5 Preparation of Sample Solution

The sample solution was prepared by accurately weighing a known quantity of tebuconazole technical sample (10 mg approx.) into a volumetric flask of 10 mL capacity, contents were dissolved in 5 mL dichloromethane and volume was made upto the mark with dichloromethane.

2.6 GC/MS Parameters

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

Instrument	:	GC/MS (Hewlett Packard-6890/5973)
Column	:	HP-5, MS; [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]
Flow rate	:	1.0 mL/min
Carrier gas	:	Helium
Injection volume	:	1 µl
Injection mode	:	Splitless (purge flow 20 mL/min)
Oven temperature	:	120 - 120 °C (hold for 6.0 minute)
		120 - 300 °C (8 °C/minute)
		300 - 300 °C (hold for 6.5 minute)
Injector temperature	:	220 °C
Transfer line temp.	:	300 °C
Mass Parameters		
Mass range	:	30 to 500 m/z
Filament delay	:	4.0 min.
Quadrupole temp	:	150 °C

2.7 Method Validation

2.7.1 Characterization/Identification

The individual solutions of each reference standards of tebuconazole active ingredient and associated impurities, standard mixture and technical sample (tebuconazole) were injected onto gas chromatograph coupled with mass spectrometer (GC/MS) and each impurity as well as active ingredient was characterized based on retention time, elution pattern, fragmentation pattern (specific ions and relative abundance) and mass (molecular ion) [**Table 1a and Fig. 1, 2a, 2b, 2c, 2d, 2e**].

2.7.2 Specificity

Like characterization, the individual and mixture solutions of reference standards, technical sample and dichloromethane (solvent) were injected onto GC/MS and specificity of the method for analysis of tebuconazole active ingredient and associated impurities was studied for any interference between the peaks of impurities and active ingredient with each other or with impurities of any standard or solvent used for preparation of solutions.

2.7.3 Linear Dynamic Range

The method linearity was established by injecting reference standard solutions of five different concentrations of tebuconazole and associated impurities in duplicate and plotting the mean areas against the concentration (ppm). The intercept (a), slope (b) and correlation co-efficient (r) values were calculated **(Table 1b)**.

2.7.4 Limit of Detection (LOD)

The limit of detection (LOD) was considered as the lowest concentration of tebuconazole or associated impurities, which can be detected with signal to noise (S/N) ratio 3 ± 0.5 : 1, by injecting various concentrations of standard solutions of tebuconazole and associated impurities onto GC/MS and calculating the S/N ratio, separately.

2.7.5 Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) was considered as the lowest concentration of tebuconazole or associated impurities, which can be quantified with signal to noise (S/N) ratio between 5 : 1 to 10 : 1, by injecting various concentrations of standard mixture solutions of tebuconazole and associated impurities onto GC/MS and calculating the S/N ratio, separately.

2.7.6 Precision (Reproducibility/ Repeatability)

The reproducibility (% RSD) of analytical method was determined by duplicate analysis of ten replicate sample preparations of the tebuconazole technical. The repeatability for tebuconazole and associated impurities was performed by five replicate injections of tebuconazole single sample onto GC/MS. The quantity of tebuconazole and associated impurities were assayed in each replicate by comparing peak area of a component in sample injection with peak area of the component in standard mixture of known concentration.

The % content, mean content, standard deviation (SD) and relative standard deviation (% RSD) or the coefficient of variation (CV) were calculated for tebuconazole and each associated impurity, separately using formula number (5), (1), (2) and (3), respectively.

$$\text{Mean content} = \frac{1}{N}\sum_{1}^{n} \text{content} \longrightarrow (1)$$

$$\text{Standard deviation (SD)} = \sqrt{\frac{\Sigma\,(\text{content} - \text{mean content})^2}{n-1}} \longrightarrow (2)$$

$$\%\ \text{RSD} = \frac{\text{Standard deviation}}{\text{Mean content}} \times 100 \longrightarrow (3)$$

2.7.7 Accuracy (% Recovery)

The accuracy (% recovery) was determined using the standard addition method. The technical sample of tebuconazole was fortified with standard mixture solution of tebuconazole and associated impurities at three fortification levels. The fortified samples were analysed by GC/MS and % recovery for tebuconazole and each associated impurity was calculated, separately at each fortification level using following formula:

$$\% \text{ Recovery (Accuracy)} = \frac{\text{Quantity recovered}}{\text{Quantity fortified}} \times 100 \quad \longrightarrow \quad (4)$$

2.8 Quantitation

Tebuconazole active ingredient and associated impurities present in the technical sample were quantified using external standard method by injecting the reference standard mixture of known concentration and comparing the responses/peak area of tebuconazole and impurities in sample and standard mixture. The active ingredient and impurities contents were calculated using following formula:

$$\text{Active ingredient / Impurity content (\%, w/w)} = \frac{R \times W_1 \times P}{R_1 \times W} \times D \quad \longrightarrow \quad (5)$$

where,

R	=	Peak area of sample
R_1	=	Mean peak area of standard
W_1	=	Weight (mg) of the standard
W	=	Weight (mg) of the sample
P	=	Purity (%, w/w) of reference standard
D	=	Dilution factor

3. Results and Discussion

The critical evaluation of GC/MS chromatograms of tebuconazole technical sample and standard mixture (**Fig. 1**) revealed that there was good resolution between peaks of tebuconazole and associated impurities without any interference between active ingredient and its impurities with each other or with any of their unknown impurity. The retention times and elution pattern of tebuconazole and associated impurities in technical sample are matching with standard mixture (**Table 1a**). Similarly, the mass spectrum of tebuconazole and all four impurities in sample showing similar fragmentation pattern and base ions as in standard mixture (**Fig. 2a, 2b, 2c, 2d, 2e**), which confirmed the identity of tebuconazole and its associated impurities.

Table 1a: Characterization / Identification data of tebuconazole and impurities.

Components	Standard Mixture			Sample			Figure
	Retention Time	Mass	Significant Fragments	Retention Time	Mass	Significant Fragments	
Tebuconazole	22.17	307	250,125,83	22.19	307	250,125,83	**2a**
Imp-1	12.24	224	139,125,57	12.24	224	139,125,57	**2b**
Imp-2	14.36	238	138,125,85	14.36	238	138,125,85	**2c**
Imp-3	19.52	273	216,129,91	19.52	273	216,129,91	**2d**
Imp-4	26.82	307	250,125,83	26.85	307	250,125,83	**2e**

Note: The impurities names have been coded, because it is considered to be commercially confidential information.

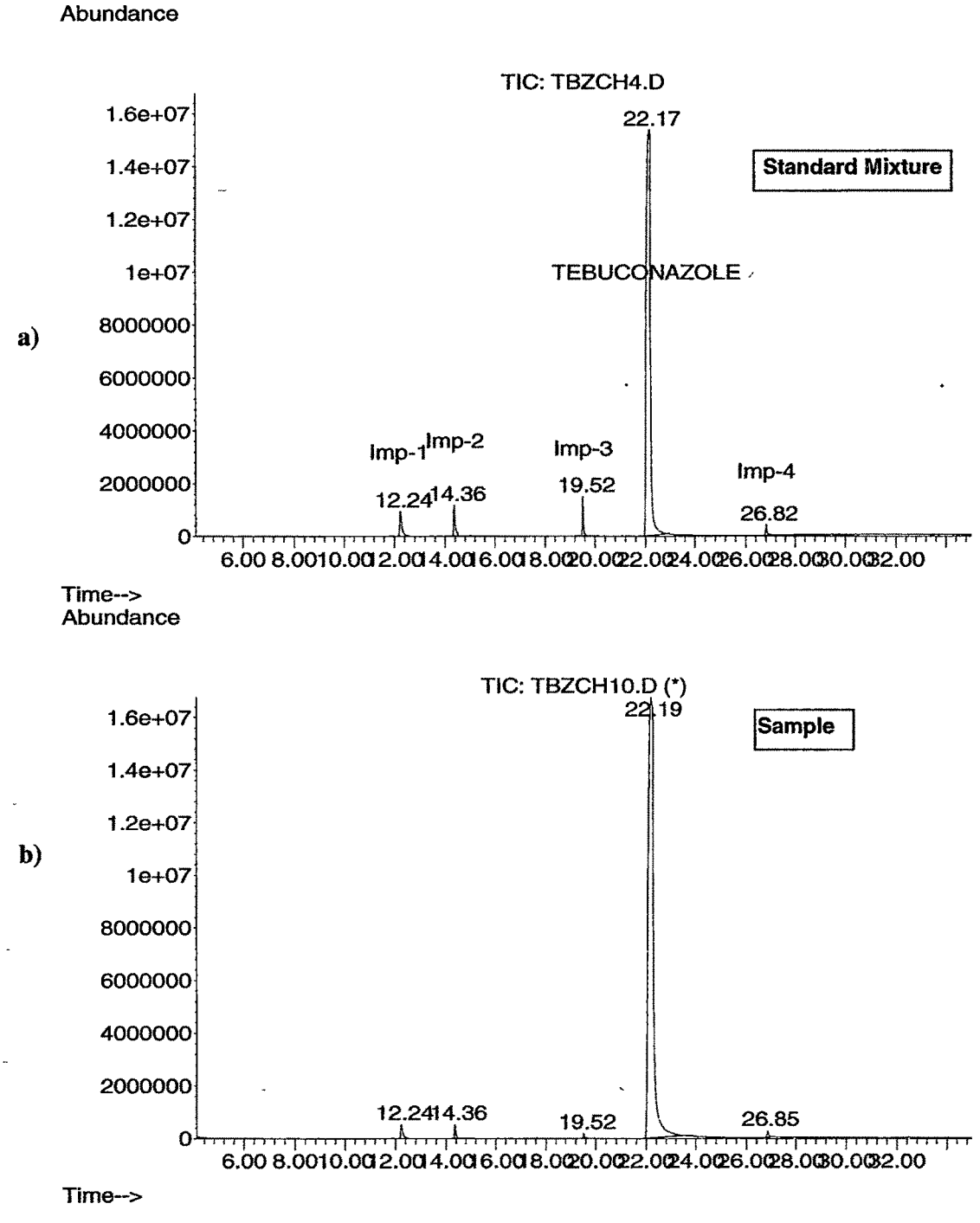
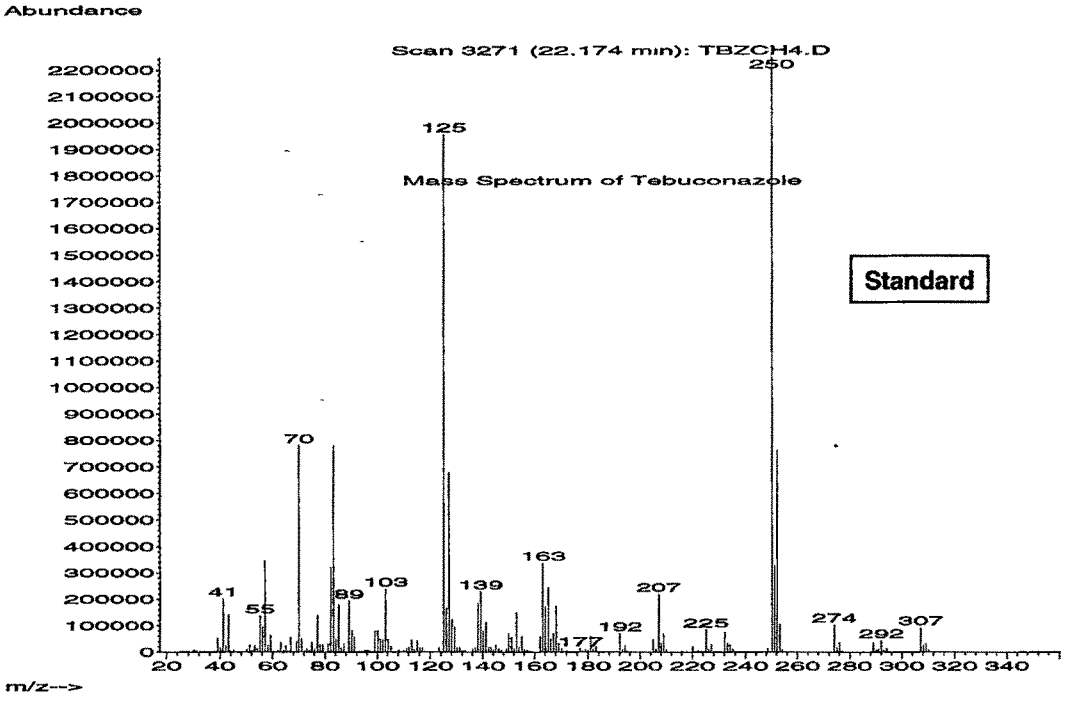


Fig. 1: Typical total ion chromatograms of a) standard mixture of tebuconazole and associated impurities, b) tebuconazole technical sample.



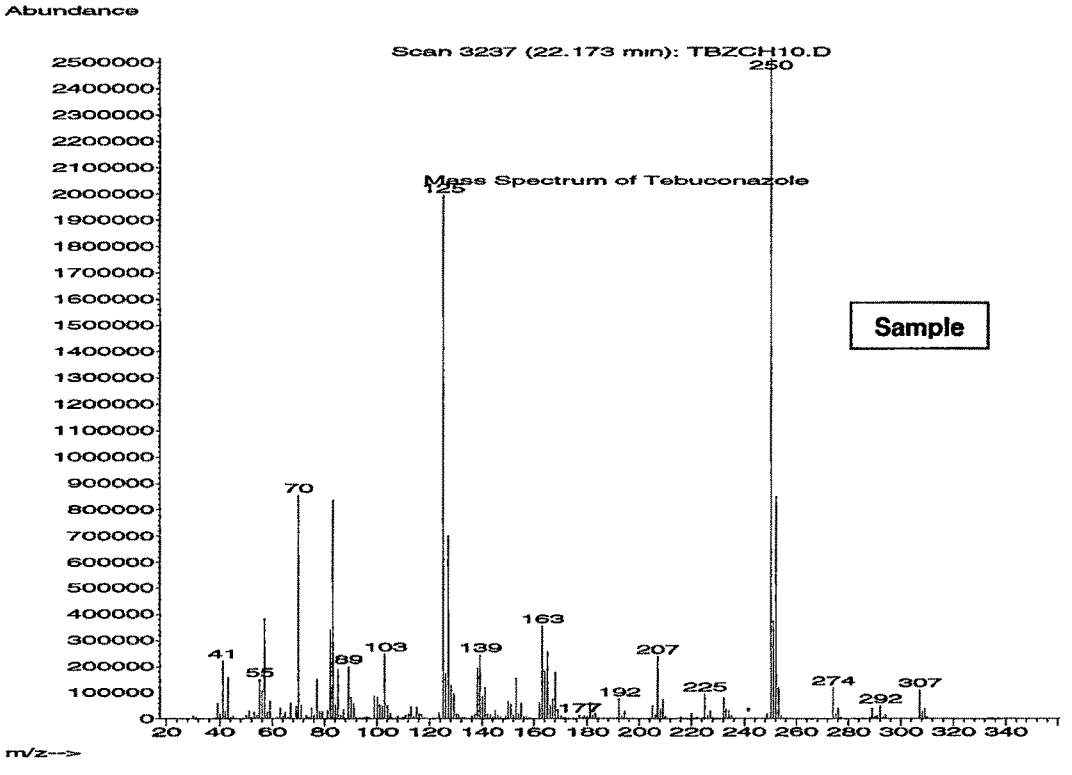
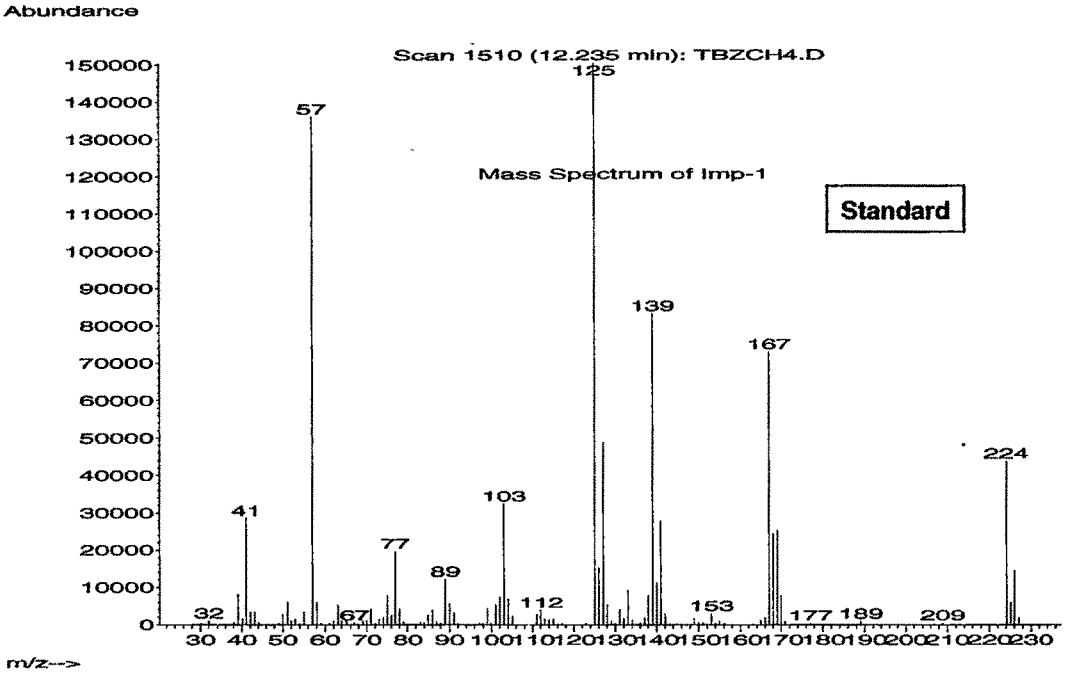


Fig. 2a : Mass spectra of tebuconazole in standard and sample injection.



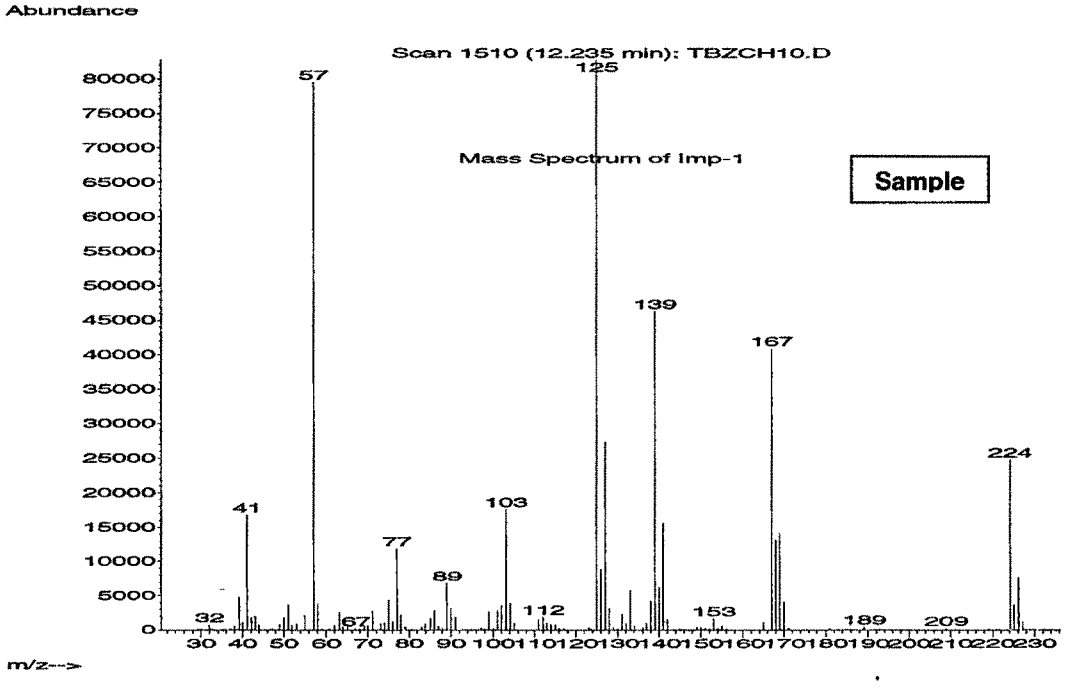
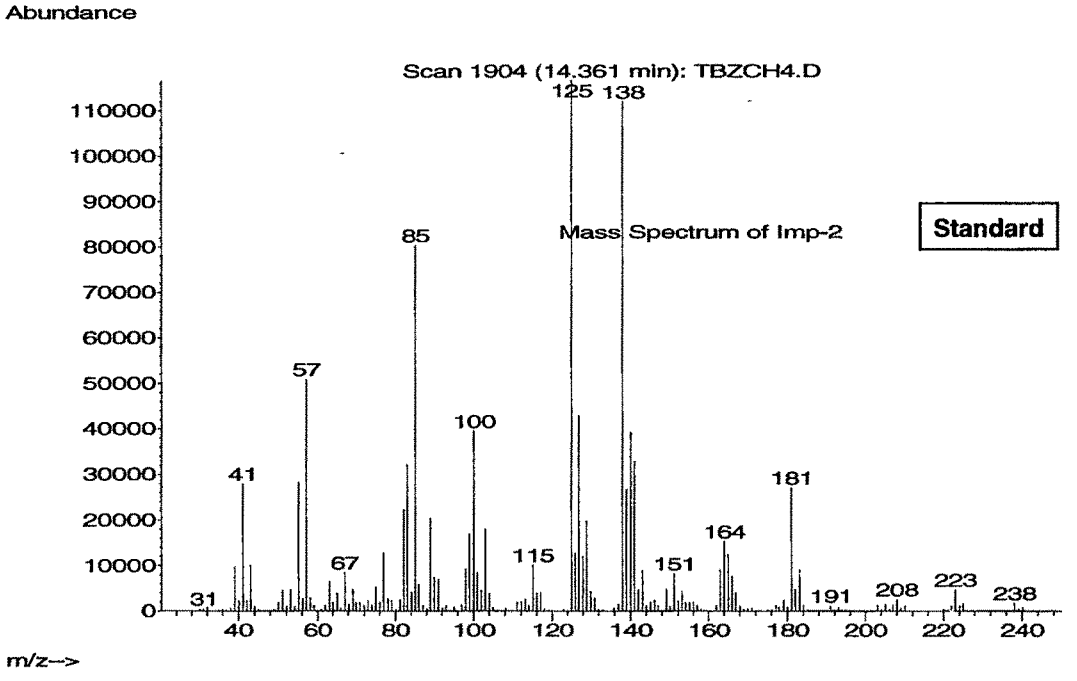


Fig. 2b : Mass spectra of impurity-1 of tebuconazole in standard and sample injection.



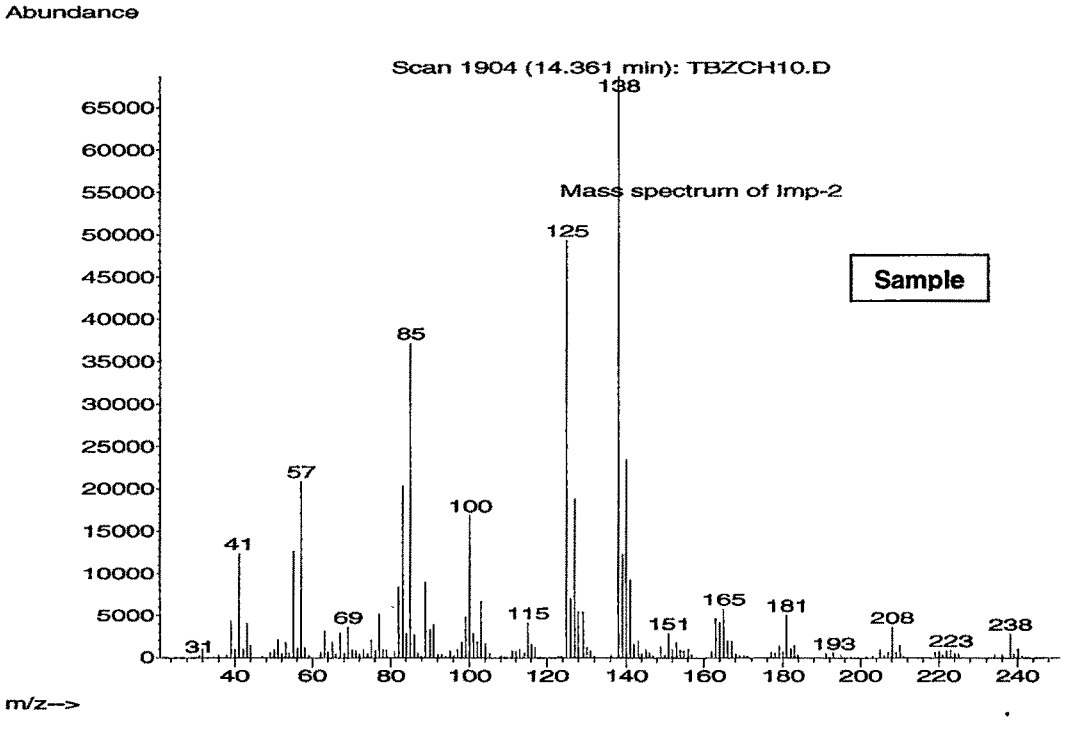
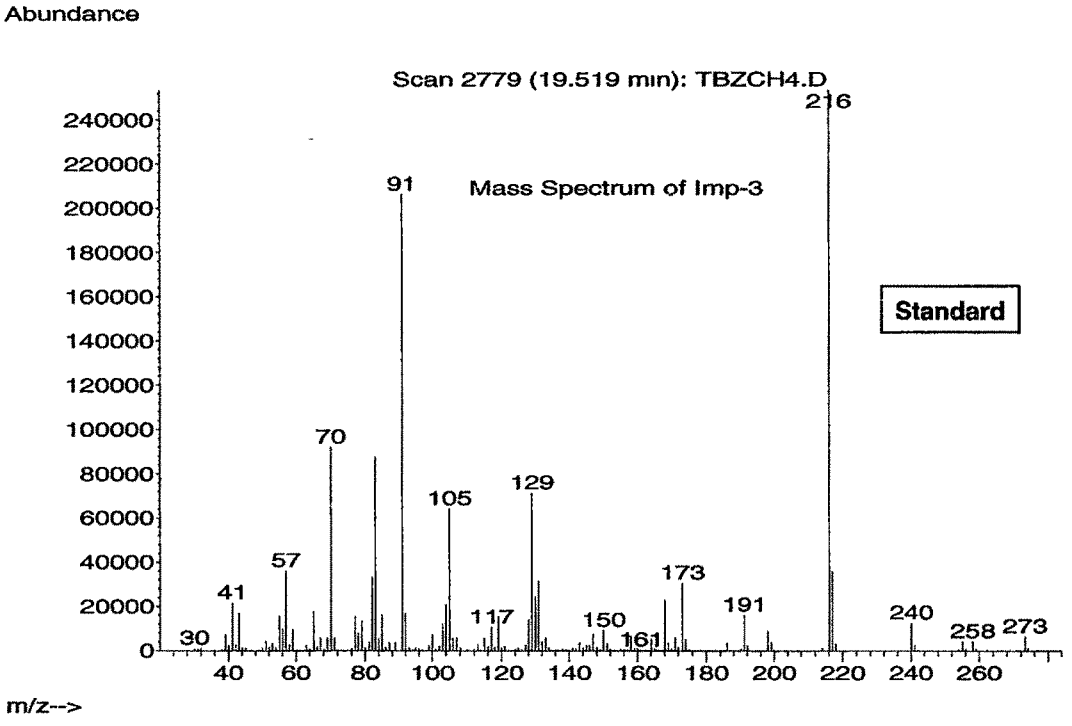


Fig. 2c: Mass spectra of impurity-2 of tebuconazole in standard and sample injection.



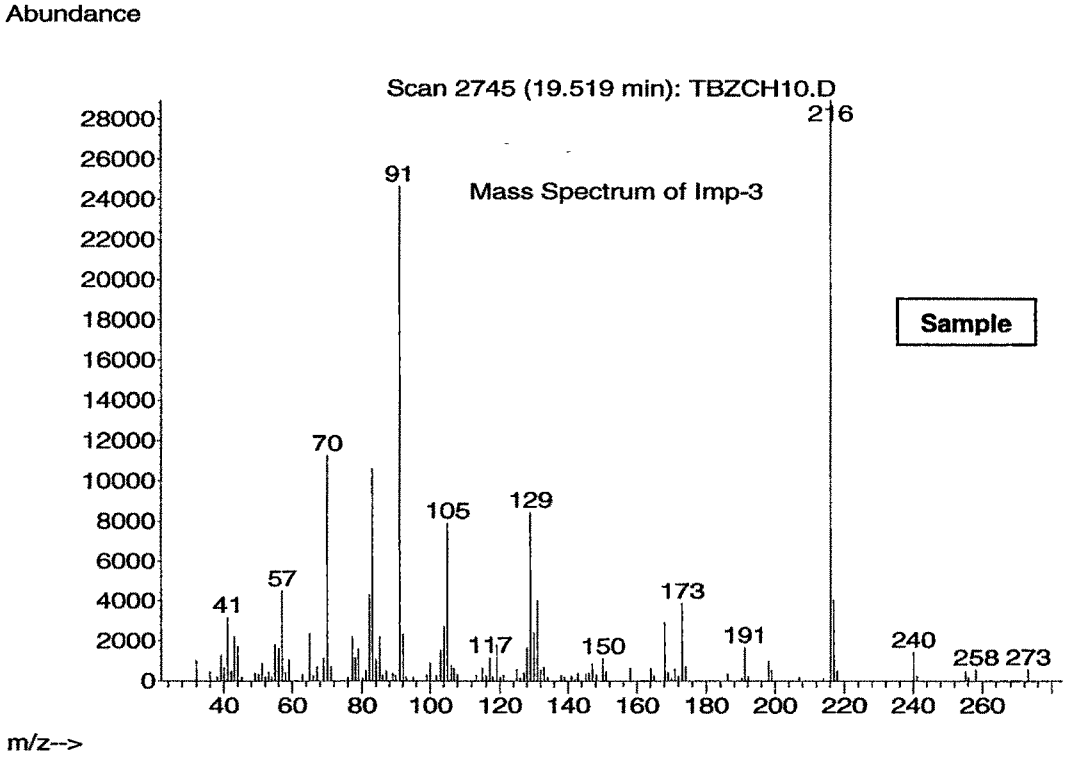


Fig. 2d : Mass spectra of impurity-3 of tebuconazole in standard and sample injection.

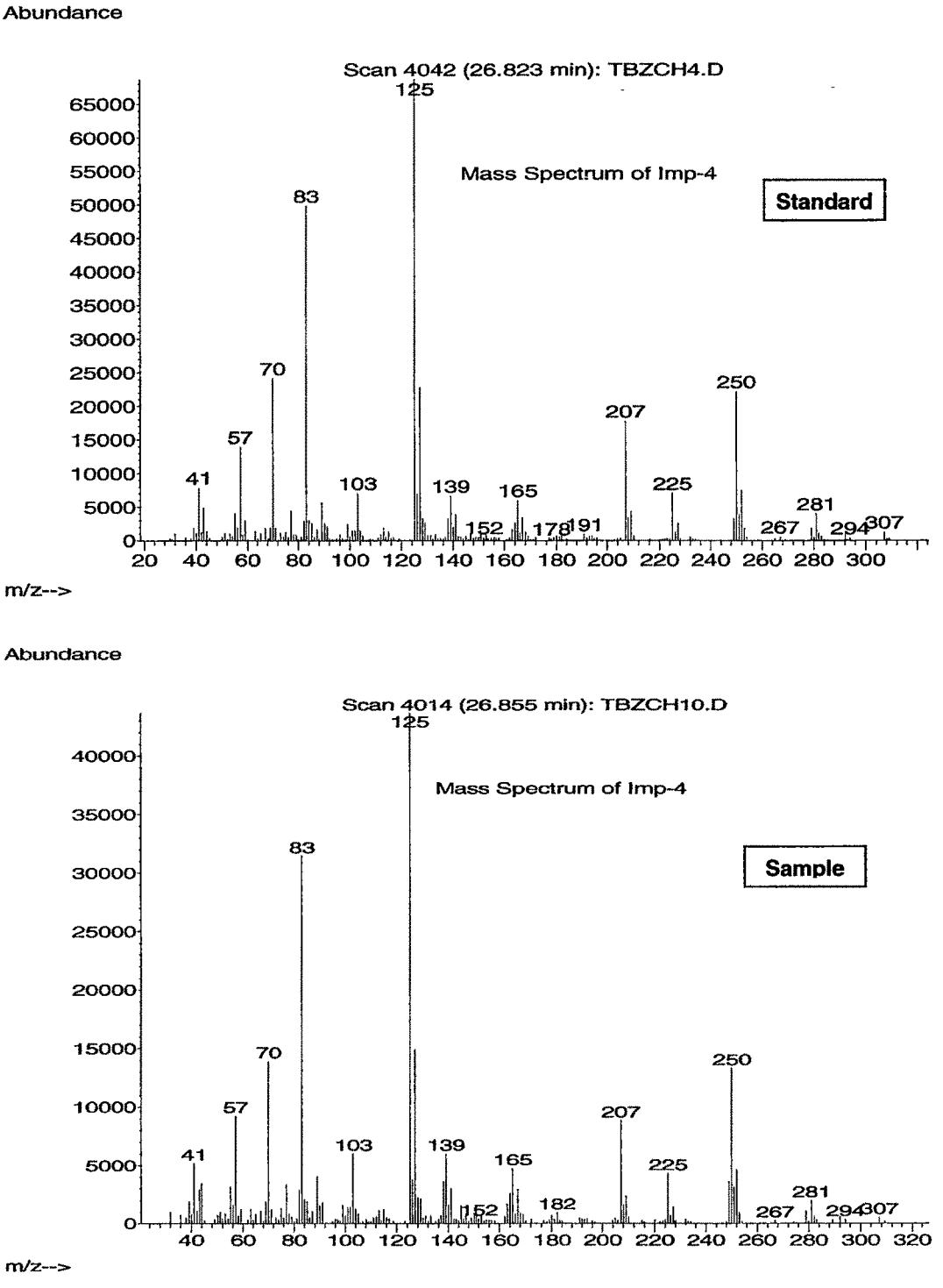


Fig. 2e : Mass spectra of impurity-4 of tebuconazole in standard and sample injection.

Before analyzing the tebuconazole technical sample for active ingredient and impurities analysis, the GC/MS method was validated. The linearity of the method was checked for tebuconazole and each of its associated impurities by analyzing the duplicate injections of five different concentrations of them and plotting the mean peak area against the concentration (**Fig. 3**). The intercept (a), slope (b) and correlation coefficient (r) values were calculated for tebuconazole and each associated impurity, separately (**Table 1b**). The correlation co-efficient (r) values ranged from 0.9991 to 0.9999, showing good linearity for tebuconazole and associated impurities.

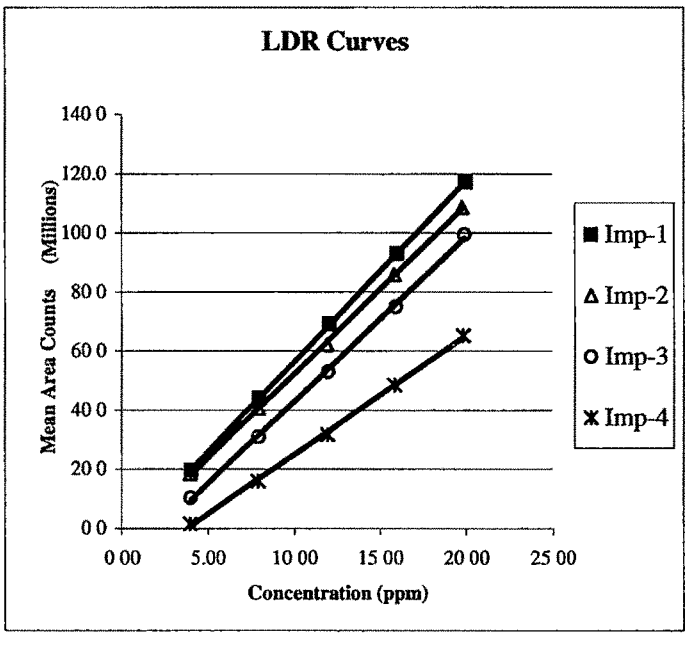


Fig. 3: Linear dynamic curves of tebuconazole impurities analyzed by GC/MS.

Table 1b: Linear dynamic range data of tebuconazole and associated impurities.

Components	Concentration Range (ppm)	Intercept (a)	Slope (b)	Correlation Coefficient (r)
Tebuconazole	199.92 – 999.58	380336248	2705671	0.9993
Imp-1	4.00 – 19.98	-4491346	6102522	0.9999
Imp-2	3.95 – 19.77	-4619134	5704369	0.9998
Imp-3	3.98 – 19.90	-12728827	5570849	0.9992
Imp-4	3.96 – 19.82	-15454215	4038797	0.9991

The limit of detection (LOD) and limit of quantitation (LOQ) were determined for active ingredient and each associated impurities (**Table 1c**), which was quite low, therefore the method can easily quantify all impurities upto 0.1% concentration level as per regulatory requirement.

The reproducibility and repeatability of method was determined by analyzing the ten sample preparations and five replicates of single sample, respectively. The relative standard deviation, % RSD (coefficient of variation, CV), calculated for tebuconazole and each associated impurity separately, ranged between 0.26 to 1.94% for reproducibility and 0.20 to 1.49% for repeatability (**Table 1c**). The % RSD values for tebuconazole and each associated impurities were found <2%, which are much below the prescribed tolerance limit of 2.68 (for analyte having concentration <1%) [Sanco guidelines][10], therefore no statistical calculations for F-test or F-ratio are needed.

The accuracy of method was determined by fortifying the technical sample with known quantities of tebuconazole and associated impurity standards at three different levels and analyzing the fortified samples for assay and % recovery of tebuconazole and impurities, separately. The mean % recovery was calculated for each component, which ranged from 97% to 99% (**Table 1c**).

Table 1c: GC/MS method validation results for tebuconazole and associated impurities.

Components	LOD (ppm)	LOQ (ppm)	Reproducibility		Repeatability		Mean Recovery (%)
			SD	% RSD	SD	% RSD	
Tebuconazole	0.26	0.51	0.25	0.26	0.19	0.20	98.60
Imp-1	0.13	0.25	0.011	1.75	0.007	1.11	97.89
Imp-2	0.13	0.25	0.012	1.94	0.007	1.14	98.19
Imp-3	0.25	0.50	0.007	1.70	0.006	1.49	98.03
Imp-4	0.99	1.98	0.018	1.74	0.013	1.26	98.99

The chemical composition / impurity profile of tebuconazole technical sample was determined by quantitation of tebuconazole and impurities using the validated GC/MS method. The tebuconazole and associated impurities contents were calculated by analyzing the technical sample in triplicate with standard mixture of known concentration. The mean % content (w/w) of tebuconazole was 97.11% and impurities viz., Imp-1, Imp-2, Imp-3 and Imp-4 were 0.66, 0.59, 0.47 and 1.03%, respectively (**Table 1d**). The total composition of all the components of tebuconazole technical sample obtained was 99.86%.

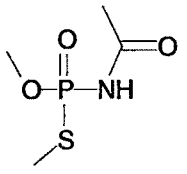
Table 1d: Chemical composition of tebuconazole technical grade sample by GC/MS.

Component	Quantitation Results (%, w/w)
Tebuconazole	97.11
Imp-1	0.66
Imp-2	0.59
Imp-3	0.47
Imp-4	1.03
Total	**99.86**

Note: The impurities names have been coded, because the composition of technical products can reveal the manufacturing process, it is considered to be commercially confidential information.

Similarly, the technical samples of various pesticides viz., acephate, chlorpyrifos, metalaxyl, ethofumesate, HCH (BHC), chlorothalonil and metribuzin were scanned by GC/MS, their impurities were identified by studying the routes of synthesis in the manufacturing process, including main and side reactions as well as possible impurities in the raw materials. Further, the identification and estimation of all associated impurities in technical pesticides were performed by developing and validating the appropriate GC/MS analytical methods for impurity profile analysis of each of the technical pesticides (**Fig. 3 to 9 and Table 2 to 8**).

3.1 Impurity Profile Analysis of Acephate Technical Sample by GC/MS



Common Name : Acephate
C A Name : N-[methoxy(methylthio)phosphinyl] acetamide
CAS No. : [030560-19-1]
Molecular Formula : $C_4H_{10}NO_3PS$
Molecular Weight : 183.2
Melting Point : 88-90 °C
Description : Colourless solid

Table 2: Chemical composition of acephate by GC/MS.

Components	Standard Mixture			Sample		
	R.T.	Mass	Significant Fragments	R.T.	Mass	Significant Fragments
Acephate	13.38	183	136, 94, 42	13.43	183	136, 94, 42
Imp-1	4.06	59	59, 44, 43	4.01	59	59, 44, 43
Imp-2	7.94	156	156, 126, 93	7.95	156	156, 126, 93
Imp-3	9.49	156	156, 110, 79	9.49	156	156, 110, 79
Imp-4	10.88	141	141, 95, 94	10.86	141	141, 95, 94

3.1.1 GC/MS Operating Conditions for Acephate Analysis

Instrument : GC/MS (Hewlett Packard-6890/5973)
Column : HP-5, MS; [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]
Oven temp. : 50 °C (hold for 3.0 min) to 280 °C @ 10 °C/min (hold for 1.0 min)
Injector temp. : 220 °C
Injection mode : Splitless (purge flow 20 mL/min)
Injection volume : 1 µl
Carrier gas : Helium
Carrier gas flow : 1.0 mL/min
Transfer line temp. : 280 °C
Mass range : 30 to 550 m/z
Filament delay : 4.0 min.
Quadrupole temp : 150 °C

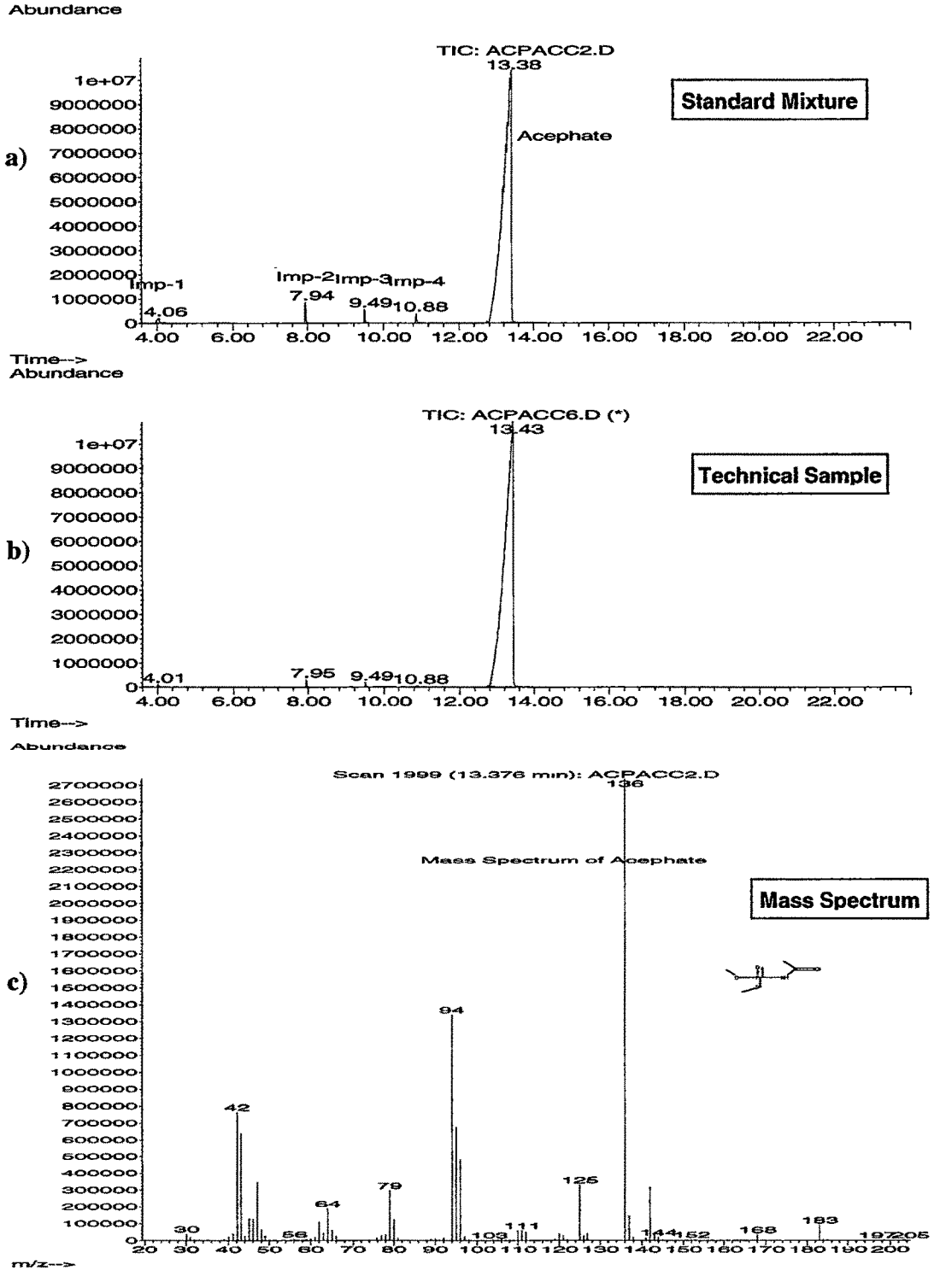
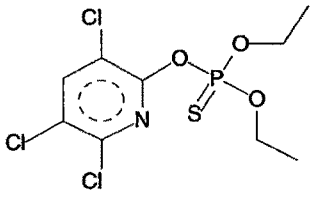


Fig. 3: Typical total ion chromatograms of a) standard mixture of acephate and associated impurities, b) acephate technical sample, c) mass spectrum of acephate.

3.2 Impurity Profile Analysis of Chlorpyrifos Technical Sample by GC/MS



Common Name : Chlorpyrifos
C A Name : O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate
CAS No. : [002921-88-2]
Molecular Formula : $C_9H_{11}Cl_3NO_3PS$
Molecular Weight : 350.6
Melting Point : 42-43.5 °C
Description : Colourless powder with a mild mercaptan odour

Table 3: Chemical composition of chlorpyrifos by GC/MS.

Components	Standard Mixture			Sample		
	R.T.	Mass	Significant Fragments	R.T.	Mass	Significant Fragments
Chlorpyrifos	20.18	351	314,286,258,197	20.17	351	314,286,258,197
Imp-1	9.01	188	188,144,132,116	9.01	188	188,144,132,116
Imp-2	12.90	217	217,215,182,180	12.91	217	217,215,182,180
Imp-3	14.48	198	197,169,134,107	14.45	198	197,169,134,107
Imp-4	16.98	322	322,266,202,174	16.98	322	322,266,202,174

3.2.1 GC/MS Operating Conditions for Chlorpyrifos Analysis

Instrument : GC/MS (Hewlett Packard-6890/5973)
Column : HP-5, MS; [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]
Oven temp. : 50 °C (hold for 3.0 min) to 280 °C @ 10 °C/min (hold for 1.0 min)
Injector temp. : 220 °C
Injection mode : Splitless (purge flow 20 mL/min)
Injection volume : 1 µl
Carrier gas : Helium
Carrier gas flow : 1.0 mL/min
Transfer line temp. : 280 °C
Mass range : 30 to 550 m/z
Filament delay : 4.0 min.
Quadrupole temp : 150 °C

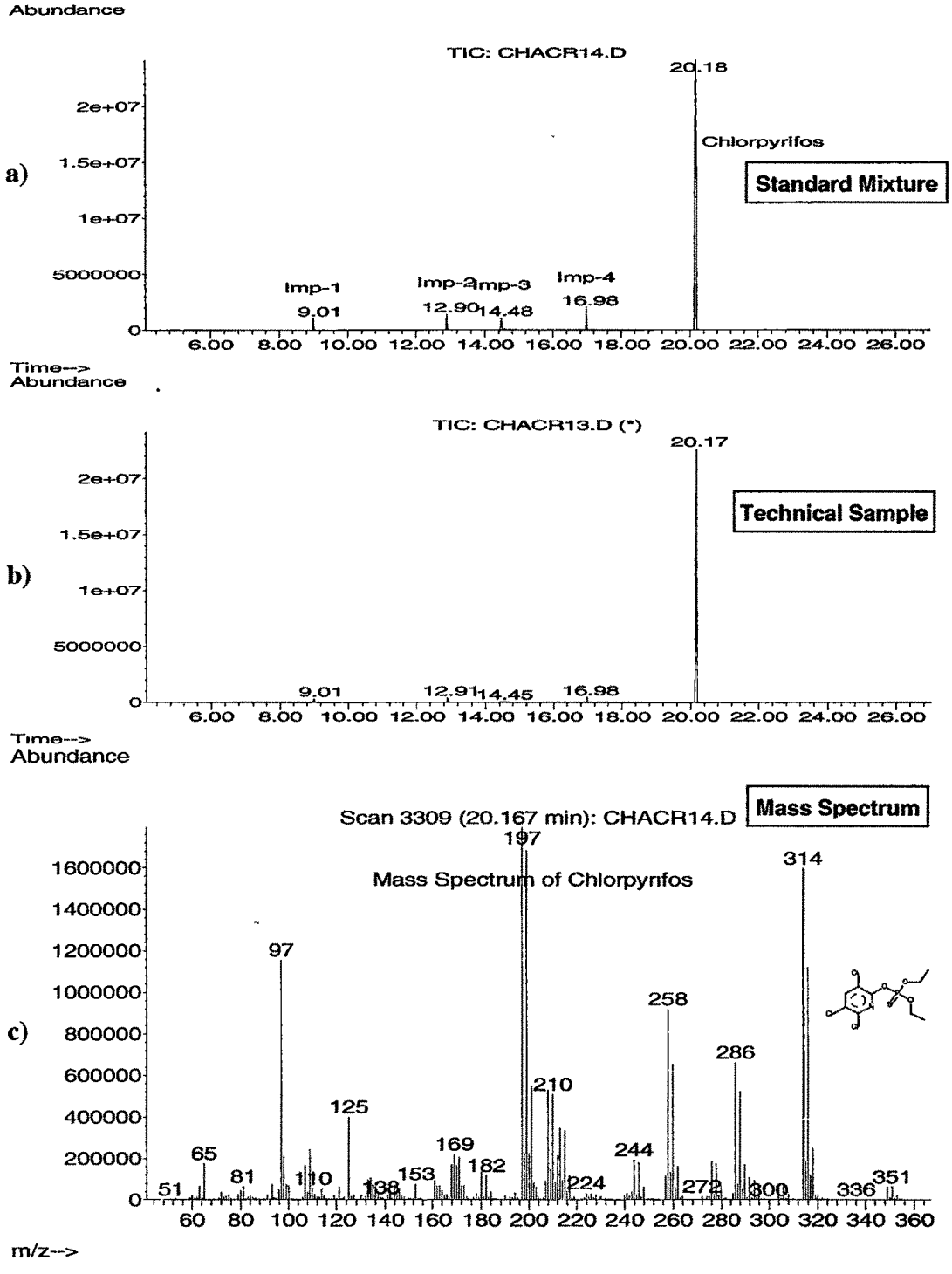
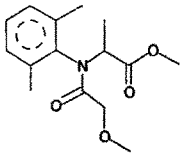


Fig. 4: Typical total ion chromatograms of a) standard mixture of chlorpyrifos and associated impurities, b) chlorpyrifos technical sample, c) mass spectrum of chlorpyrifos.

3.3 Impurity Profile Analysis of Metalaxyl Technical Sample by GC/MS



Common Name : Metalaxyl
C A Name : Methyl-N-(2,6-dimethylphenyl)-N-(methoxyacetyl)- DL-alaninate
CAS No. : [057837-19-1]
Molecular Formula : $C_{15}H_{21}NO_4$
Molecular Weight : 279.3
Melting Point : 63.5-72.3 °C
Description : Fine, white powder.

Table 4: Chemical composition of metalaxyl by GC/MS.

Components	Standard Mixture			Sample		
	R.T.	Mass	Significant Fragments	R.T.	Mass	Significant Fragments
Metalaxyl	15.48	279	249,234,220,192	15.48	279	249,234,220,192
Imp-1	12.30	207	207,148,132	12.30	207	207,148,132
Imp-2	13.28	193	193,148,134,120	13.27	193	193,148,134,120
Imp-3	15.80	283	234,224.148	15.80	283	234,224.148

3.3.1 GC/MS Operating Conditions for Metalaxyl Analysis

Instrument : GC/MS (Hewlett Packard-6890/5973)
Column : HP-5, MS; [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]
Oven temp. : 50 °C (hold for 3.0 min) to 280 °C @ 10 °C/min (hold for 1.0 min)
Injector temp. : 220 °C
Injection mode : Splitless (purge flow 20 mL/min)
Injection volume : 1 µl
Carrier gas : Helium
Carrier gas flow : 1.0 mL/min
Transfer line temp. : 280 °C
Mass range : 30 to 550 m/z
Filament delay : 4.0 min.
Quadrupole temp : 150 °C

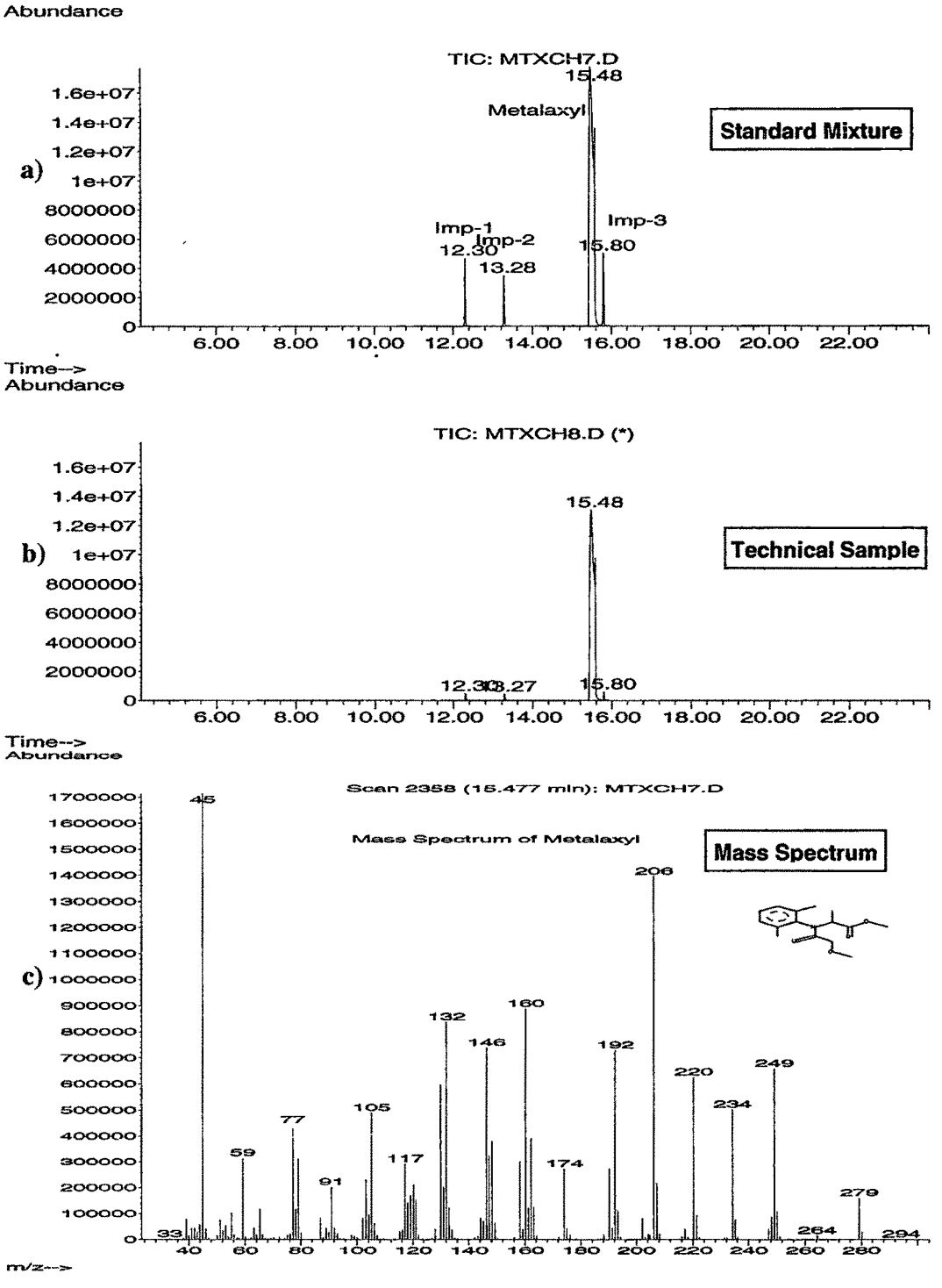
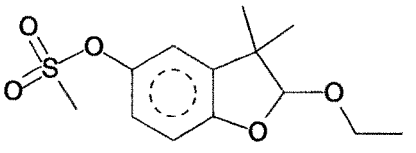


Fig. 5: Typical total ion chromatograms of a) standard mixture of metalaxyl and associated impurities, b) metalaxyl technical sample, c) mass spectrum of metalaxyl.

3.4 Impurity Profile Analysis of Ethofumesate Technical Sample by GC/MS



Common Name : Ethofumesate
C A Name : 2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate
CAS No. : [026225-79-6]
Mol. Formula : $C_{13}H_{18}O_5S$
Mol. Weight : 286.3
Melting Point : 70-72 °C
Description : Light brown crystalline solid.

Table 5: Chemical composition of ethofumesate by GC/MS.

Components	Standard Mixture			Sample		
	R.T.	Mass	Significant Fragments	R.T.	Mass	Significant Fragments
Ethofumesate	18.68	286	286,207,179,161	18.70	286	286,207,179,161
Imp-1	7.14	124	109,97,79	-	-	-
Imp-2	11.03	152	111,80,56	-	-	-
Imp-3	16.98	208	208,161,147,137	16.98	208	208,161,147,137
Imp-4	18.86	258	258,229,179,161	18.88	258	258,229,179,161
Imp-5	19.09	266	266,187,109,79	19.06	266	266,187,109,79
Imp-6	20.61	327	327,249,248	20.60	327	327,249,248

3.4.1 GC/MS Operating Conditions for Ethofumesate Analysis

Instrument : GC/MS (Hewlett Packard-6890/5973)
Column : HP-5, MS; [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]
Oven temp. : 50 °C (hold for 3.0 min) to 280 °C @ 10 °C/min (hold for 1.0 min)
Injector temp. : 220 °C
Injection mode : Splitless (purge flow 20 mL/min)
Injection volume : 1 µl
Carrier gas : Helium
Carrier gas flow : 1.0 mL/min
Transfer line temp. : 280 °C
Mass range : 30 to 550 m/z
Filament delay : 4.0 min.
Quadrupole temp : 150 °C

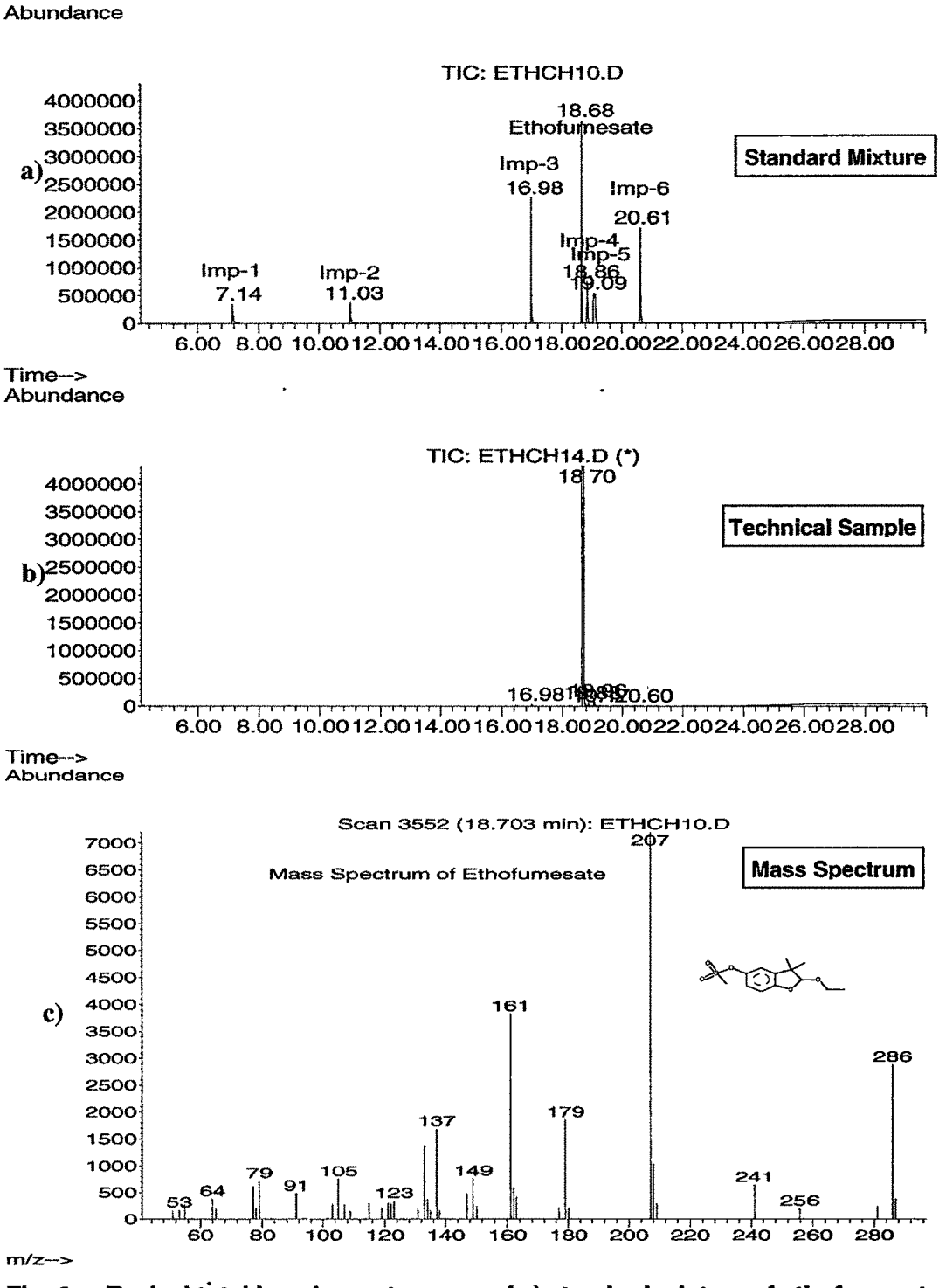
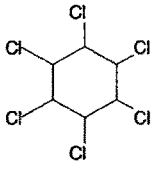


Fig. 6: Typical total ion chromatograms of a) standard mixture of ethofumesate and associated impurities, b) ethofumesate technical sample, c) mass spectrum of ethofumesate.

3.5 Impurity Profile Analysis of HCH Technical Sample by GC/MS



Common Name	:	HCH
C A Name	:	1,2,3,4,5,6-hexachlorocyclohexane (mixed isomers)
CAS No.	:	[000319-85-7]
Molecular Formula	:	$C_6H_6Cl_6$
Molecular Weight	:	290.8
Melting Point	:	112.9 °C
Description	:	Off-white to brown powder with a musty smell.

Table 6: Chemical composition of HCH by GC/MS.

Components	Code	Standard Mixture			Sample		
		R.T.	Mass	Significant Fragments	R.T.	Mass	Significant Fragments
HCH	BHC	12.36	291	219,181,111	12.38	291	219,181,111
γ-2,3,4,5,6-= pentachlorocyclohexane	Imp-1	10.64	253	219,181,146	10.64	253	219,181,146
β-HCH	Imp-2	13.33	291	219,181,109	13.29	291	219,181,109
γ-HCH	Imp-3	13.49	291	219,181,109	13.45	291	219,181,109
δ-HCH	Imp-4	14.50	291	219,181,109	14.51	291	219,181,109
ε-HCH	Imp-5	14.81	291	219,181,109	14.81	291	219,181,109

3.5.1 GC/MS Operating Conditions for HCH Analysis

Instrument	:	GC/MS (Hewlett Packard-6890/5973)
Column	:	HP-5, MS; [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]
Oven temp.	:	50 °C (hold for 3.0 min) to 280 °C @ 10 °C/min (hold for 1.0 min)
Injector temp.	:	220 °C
Injection mode	:	Splitless (purge flow 20 mL/min)
Injection volume	:	1 µl
Carrier gas	:	Helium
Carrier gas flow	:	1.0 mL/min
Transfer line temp.	:	280 °C
Mass range	:	30 to 550 m/z
Filament delay	:	4.0 min.
Quadrupole temp	:	150 °C

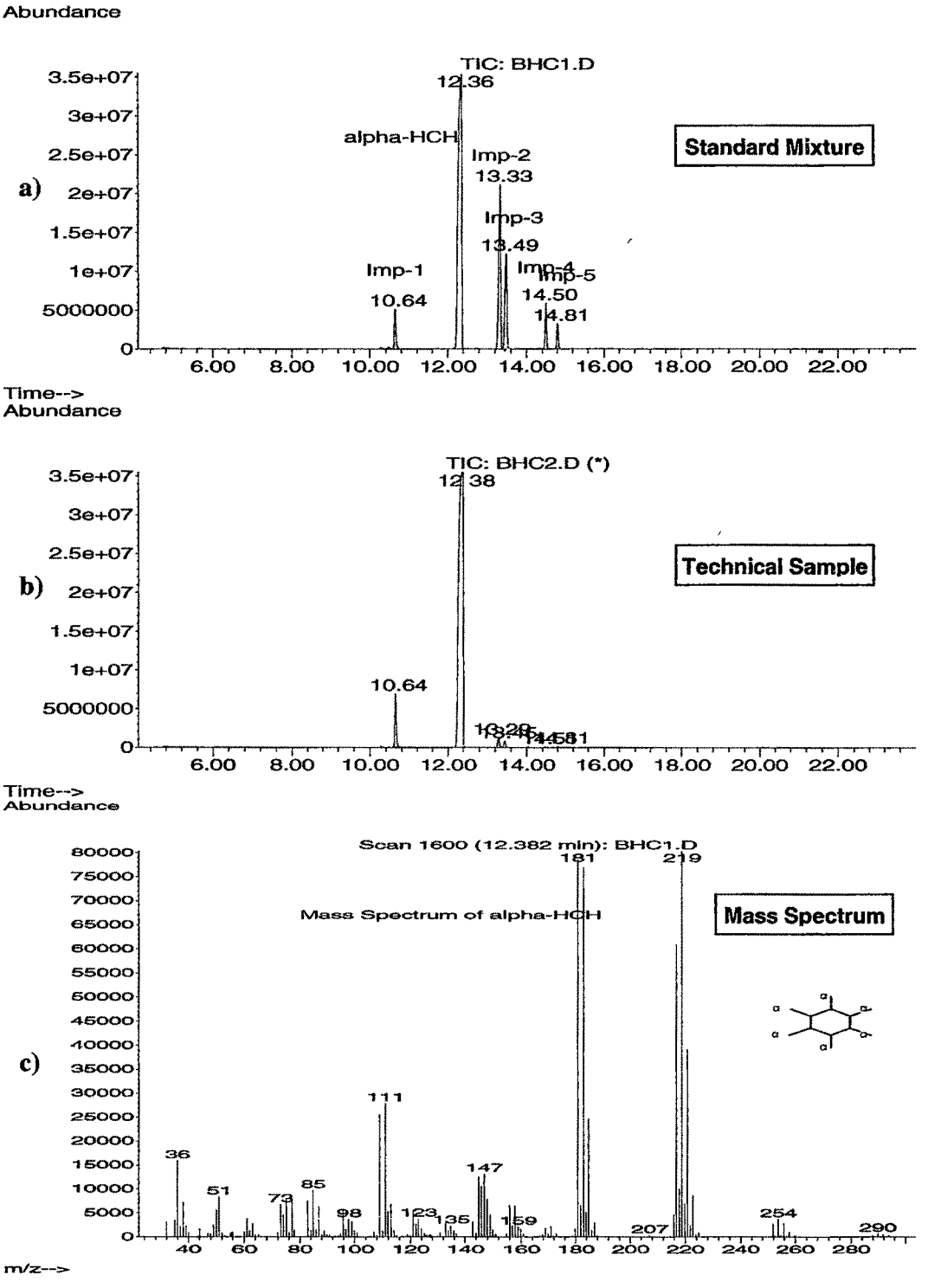
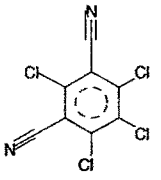


Fig. 7: Typical total ion chromatograms of a) standard mixture of HCH active ingredient and associated impurities, b) HCH technical grade sample, c) mass spectrum of HCH.

3.6 Impurity Profile Analysis of Chlorothalonil Technical Sample by GC/MS



Common Name	:	Chlorothalonil
C A Name	:	2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile
CAS No.	:	[001897-45-6]
Molecular Formula	:	$C_8Cl_4N_2$
Molecular Weight	:	265.9
Melting Point	:	252.1 °C
Description	:	Colourless odourless crystals.

Table 7: Chemical composition of chlorothalonil by GC/MS.

Components	Standard Mixture			Sample		
	R.T.	Mass	Significant Fragments	R.T.	Mass	Significant Fragments
Chlorothalonil	15.70	266	266,264,229	15.80	266	266,264,229
Imp-1	14.76	275	275,273,240	14.77	275	275,273,240
Imp-2	15.20	266	266,264,229	15.29	266	266,264,229
Imp-3	16.30	266	266,264,229	-	-	-

3.6.1 GC/MS Operating Conditions for Chlorothalonil Analysis

Instrument	:	GC/MS (Hewlett Packard-6890/5973)
Column	:	HP-5, MS; [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]
Oven temp.	:	50 °C (hold for 3.0 min) to 280 °C @ 10 °C/min (hold for 1.0 min)
Injector temp.	:	220 °C
Injection mode	:	Splitless (purge flow 20 mL/min)
Injection volume	:	1 µl
Carrier gas	:	Helium
Carrier gas flow	:	1.0 mL/min
Transfer line temp.	:	280 °C
Mass range	:	30 to 550 m/z
Filament delay	:	4.0 min.
Quadrupole temp	:	150 °C

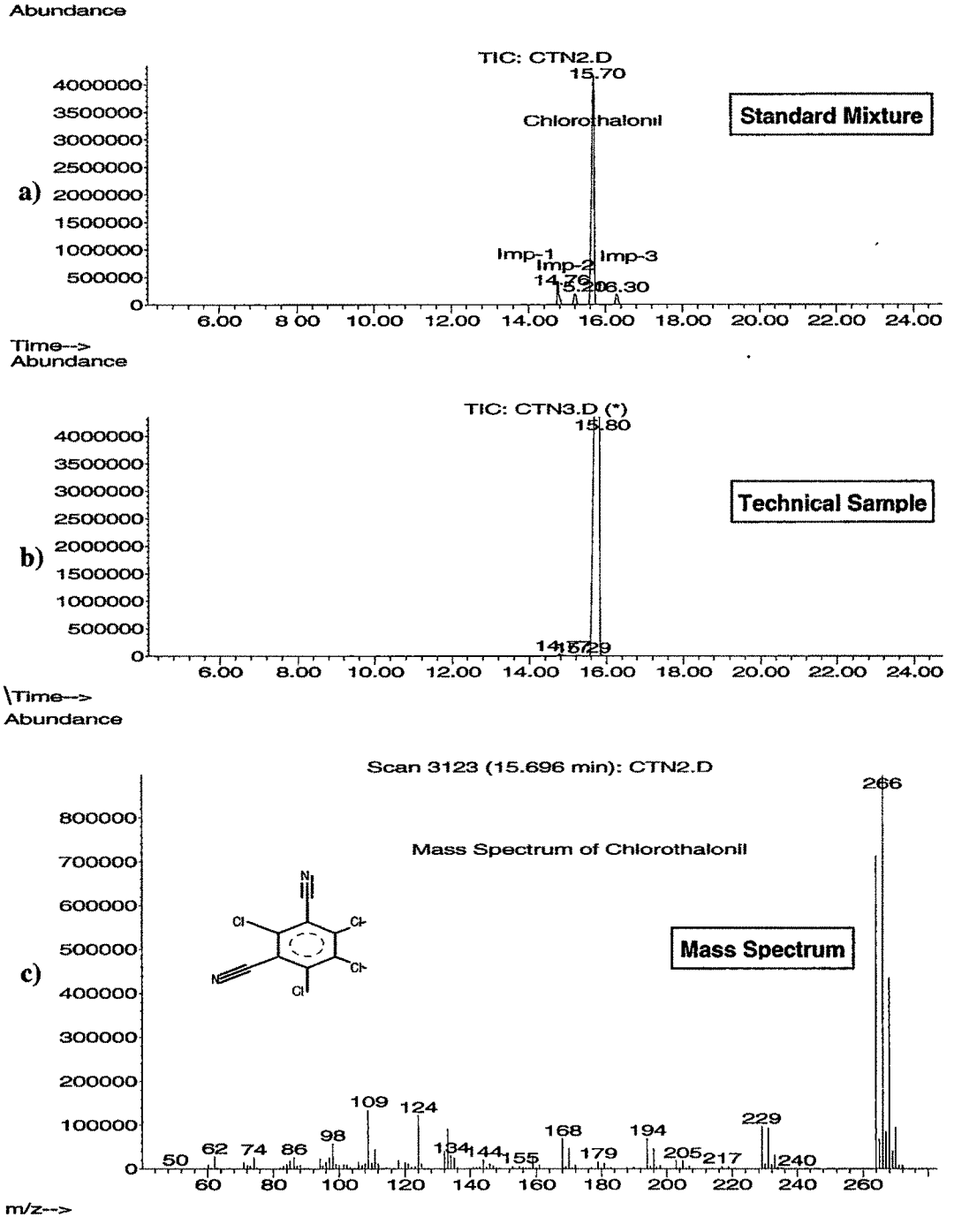
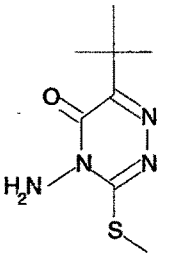


Fig. 8: Typical total ion chromatograms of a) standard mixture of chlorothalonil and associated impurities, b) chlorothalonil technical sample, c) mass spectrum of chlorothalonil.

3.7 Impurity Profile Analysis of Metribuzin Technical Sample by GC/MS



Common Name : Metribuzin

C A Name : 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one

CAS No. : [021087-64-9]

Mol. Formula : $C_8H_{14}N_4OS$

Molecular Weight : 214.3

Melting Point : 126.2 °C

Description : White crystals with a weak characteristic odour.

Table 8: Chemical composition of metribuzin by GC/MS.

Components	Standard Mixture			Sample		
	R.T.	Mass	Significant Fragments	R.T.	Mass	Significant Fragments
Metribuzin	16.11	214	198,182,171,144	16.12	214	198,182,171,144
Imp-1	14.71	214	214,198,186,171	14.70	214	214,198,186,171
Imp-2	14.91	228	198,117,74	14.90	228	198,117,74

3.7.1 GC/MS Operating Conditions for Metribuzin Analysis

Instrument : GC/MS (Hewlett Packard-6890/5973)

Column : HP-5, MS; [30 m x 0.25 mm (i.d.) x 0.25 µm film thickness]

Oven temp. : 50 °C (hold for 3.0 min) to 280 °C @ 10 °C/min (hold for 1.0 min)

Injector temp. : 220 °C

Injection mode : Splitless (purge flow 20 mL/min)

Injection volume : 1 µl

Carrier gas : Helium

Carrier gas flow : 1.0 mL/min

Transfer line temp. : 280 °C

Mass range : 30 to 550 m/z

Filament delay : 4.0 min.

Quadrupole temp : 150 °C

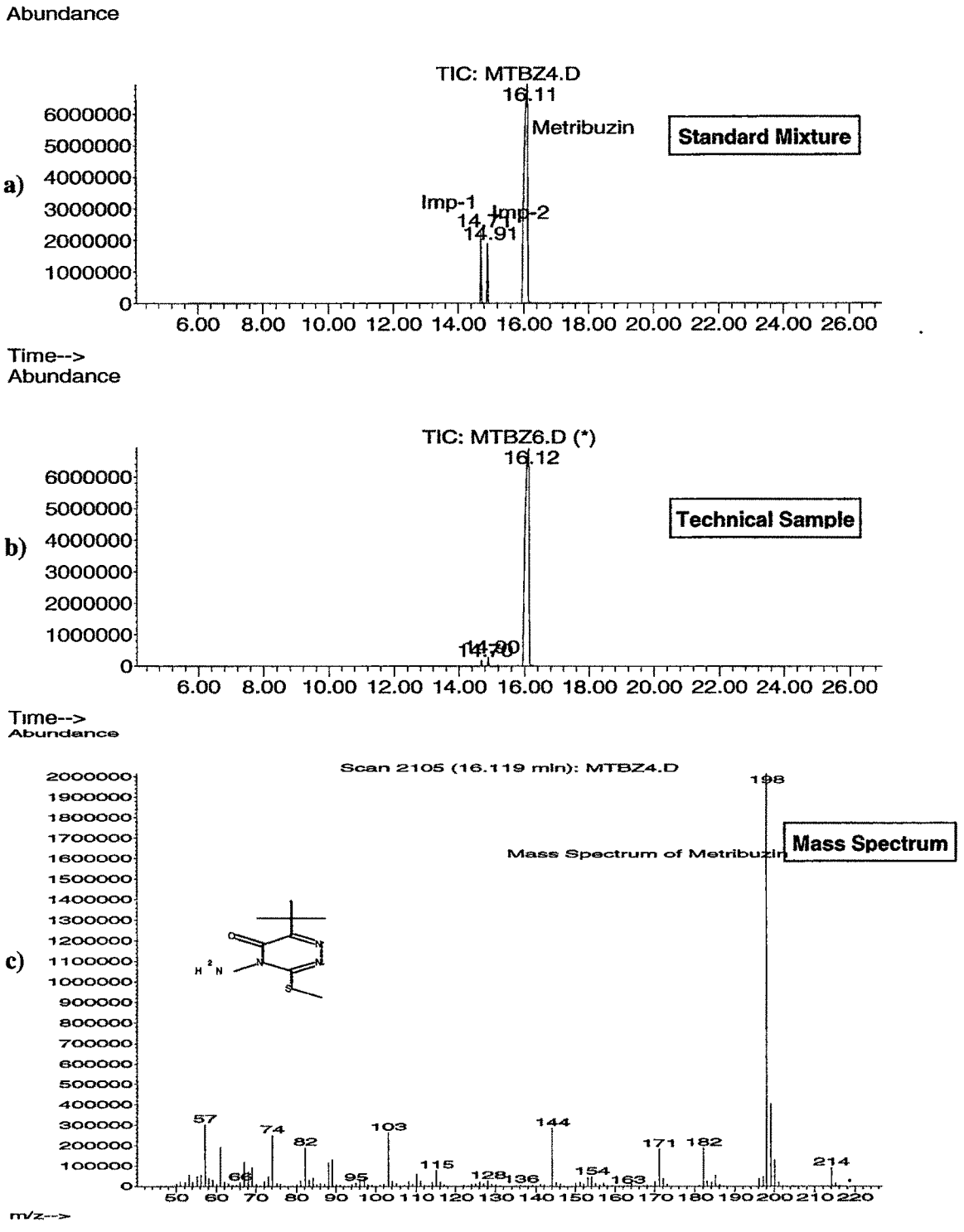


Fig. 9: Typical total ion chromatograms of a) standard mixture of metribuzin and associated impurities, b) metribuzin technical sample, c) mass spectrum of metribuzin.

4. Conclusions

Manufacturers of pesticides should regularly check the quality of pesticide products marketed and the concentration of the relevant impurities to assure the chemical composition of technical grade products complies with quality specification. As per regulatory requirements, all the impurities of a technical compound which are >0.1%, need to be identified and quantified. Therefore, development of appropriate validated analytical methods is of vital importance for impurity profile analysis of technical compounds. An attempt has been made to develop the suitable GC/MS analytical methods for impurity profile analysis of eight technical pesticides viz., tebuconazole, acephate, chlorpyrifos, metalaxyl, ethofumesate, chlorothalonil, HCH, and metribuzin. The methods were validated before estimation of active ingredients and associated impurities of technical grade pesticides. The proposed analytical methods can be employed for the routine quality-monitoring program.

5. References

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