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Persistence of Indoxacarb on Cauliflower (*Brassica oleracea* var. *botrytis*. L.) and Its Risk Assessment

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Abstract

A rapid, simple and an efficient method for the determination of indoxacarb in cauliflower and soil samples was developed and validated using QuEChERS technique (Quick, Easy, Cheap, Effective, Rugged and Safe). Recoveries at four different spiking concentrations of 0.01, 0.05, 0.1 and 0.2 mg·kg^{-1} ranged from 87% to 96% were achieved with good repeatability and RSD of 1% - 6%. The average initial deposits of 0.23 and 0.45 mg·kg^{-1} were observed after last application of indoxacarb @ 52.2 and 104.4 g. a.i. ha^{-1} at recommended and double the recommended dosages, respectively. The residues in cauliflower dissipated below its LOQ of 0.01 mg·kg^{-1} after 7 days and its half-life periods were observed to be 1.12 and 1.31 days, respectively, at single and double the dosages. Keeping in view 80 g consumption of cauliflower curds per day for a 55 kg person, theoretical maximum residue contribution (TMRC) of indoxacarb when calculated from maximum residues observed on 0 day samples at recommended and double the recommended dosages, respectively, were found to be 20.8 and 36.8 µg in comparison to its acceptable daily intake (ADI) of 550 µg, which is quite safe.

Keywords: Cauliflower, Indoxacarb, MRL, QuEChERS, Residue

1. Introduction

Indoxacarb, Methyl 7-Chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4-(trifluoromethoxy)phenyl]amino]carbonyl] indeno[1,2-e][1,3,4]oxadiazine-4a(3*H*)-carboxylate, is a non-systemic, synthetic organophosphate replacement insecticide with broad spectrum activity [1,2]. This relatively new pesticide was developed by E. I. du Pont de Nemours and Company and was registered as Avaunt and Steward in California, January 2001 [2]. Indoxacarb, technical material is a white powered solid and it is formulated as a wettable granule and soluble concentrate [3]. It is an oxadia zine insecticide that blocks the sodium channels in insect nerve cells, causing lepidopteran larvae to stop feeding within 4 hours, become paralyzed and die within 2 to 5 days [4]. Indoxacarb is a highly effective new insecticide has low side effect on non target insects [5,6] and allows most predators and immature wasp parasites to survive [7,8] however, the wet resi dues of indoxacarb are toxic to bees and adult wasp parasites. It is considered as a reduced risk pesticide as compared to that of conventional pesticide [9].

Cauliflower is one of the most important cruciferous vegetable crops of India. It is widely cultivated throughout the sub-tropical parts of north India. In Punjab, the area under cauliflower was 5.59 thousand hactares with the production of 131.40 thousand tonnes [10]. Cauliflower is low in fat, high in dietary fibers, contains water and vitamin C, possessing a very high nutritional density [11]. It contains other glucosinolates besides sulfurophane, substances which may improve the livers ability to detoxify carcinogenic substances. A high intake of cauliflower has been found to reduce the risk of aggressive prostate cancer [12]. One of the major constraints in commercial growth of the crop is the severe damage caused to its leaves and curds by insects pests such as cabbage borer (*Hellula undalis*), leaf webber (*Crocidolomia binotalis*), diamond back moth (*Plutella xylostella*), cut worm (Lepidoptera: Noctuidae), aphids (*Lipaphis psuedobrassicae*), cabbage flea beetle (*P. cruciferae*) and tobacco caterpillar (*Spodoptera litura*) resulting in severe loss of quality and production [13,14].

Studies revealed from the field persistence test confirmed that indoxacarb were effective against *P. xylostella* [15-18]. It is highly toxic and very strong ovicidal action with LC_{50} value of 0.0064% against *S. litura* [19].

Indoxacarb has been used to control of leaf folder, fruit borer in chillies [20] tomato fruit borer [21] and cabbage looper [22] also. So, indoxacarb may play an important role in integrated pest management programmes with its novel mode of action, quick cessation of feeding, persistence under field conditions and compatibility with natural enemies [17]. Keeping in view, the present studies were undertaken to determine the residue dynamics and final residues of indoxacarb on cauliflower and to find out the safe period for harvest of the produce for consumer safety under Punjab climate conditions at different time intervals.

2. Experimental Sections

2.1. Materials

The organic solvents including acetonitrile and acetone used were of HPLC grade and GR grade respectively, purchased from Merck (Darmstadt, Germany). Sodium chloride (ACS reagent grade ≥99.9%) and sodium sulfate anhydrous (AR grade) were obtained from SD fine chemicals, Mumbai, India. Analytical-grade $MgSO_4$ (was purchased from Merck, India) were activated by heating at 400˚C in muffle furnace for 3 hrs, cooled and kept in a desiccators before use. Graphatised carbon and primary secondary amine (PSA) were obtained from Sigma Adrich and Varian, Mumbai, India, respectively.

2.2. Stock Solutions

Technical grade pesticide indoxacarb (≥99.9% purity) was purchased from Dr. Ehrestorfer Augusburg, Germany. The stock solution of indoxacarb containing 1000 $\mu g \cdot mL^{-1}$ of analyte was prepared using acetone as solvent and kept at –20˚C. Working solutions of 0.01, 0.05, 0.1, 0.5, 1.0 and 5.0 $\mu g \cdot mL^{-1}$ concentrations were prepared by serial dilutions and stored at 4˚C. These standard solutions were used for fortification of the matrices and instrument calibration purposes.

2.3. Field Trial

Field experiment was conducted during November 2009 - March 2010 following good agronomic practices at Vegetable Research Farm, Punjab Agricultural University, Ludhiana, using randomized block design (RBD) [10]. There were three replications for each treatment and the plot size of each treatment was 100 m^2 **Figure 1** .Three applications of insecticide were made starting at fruit initiation followed by second and third spraying at 10 days intervals with the help of Aspee Knapsack sprayer equipped with hollow cone nozzle. The treatments were untreated control (T_1), single dose @ 52.2 (T_2) and double dose @ 104.4 g a.i. ha^{-1} (T_3). Untreated control was sprayed with water only.

2.4. Sampling

About 5 - 6 cauliflower curds were collected at 0, 1, 3, 5, 7, 10 and 15 days after the last application of the insecticide. The samples were collected at random from each plot separately, ensuring the samples were reliable and representative. Cauliflower curd samples were packed in polyethylene bags and transported to the laboratory where they were chopped and mixed thoroughly. The soil samples of about 1 kg were collected from each plot separately after 15 days following the last application. Pebbles and other unwanted materials were removed manually from field soil, air-dried, powered and pass through a 3 mm sieve to achieve uniform mixing. Soil samples were collected separately from 15 sites of each treated plot with the help of tube auger at a depth of about 10 - 15 cm; the soil from the 15 sites were pooled and sieved, and extraneous matter, including stones/ pebbles, were removed. The texture and characteristics

Passage				
CONTROL PLOT	**WATER CHHANNEL**	**DOUBLE DOSE (**Indoxacarb @ 104.4 g a.i. ha^{-1}**)**	**WATER CHHANNEL**	**RECOMMENDED DOSE (**Indoxacarb @ 52.2 g a.i. ha^{-1}**)**
RECOMMENDED DOSE (Indoxacarb @ 52.2 g a.i. ha^{-1}**)**		**CONTROL PLOT**		**DOUBLE DOSE (**Indoxacarb @ 104.4 g a.i. ha^{-1}**)**
DOUBLE DOSE (Indoxacarb @ 104.4 g a.i. ha^{-1}**)**		**RECOMMENDED DOSE (**Indoxacarb @ 52.2 g a.i. ha^{-1}**)**		**CONTROL PLOT**
Water channel				

Figure 1. Layout of the field experiment.

of field soil were sand 78.0%, slit 10.2%, clay 11.8%, organic carbon 0.30%, EC 0.30 dsm^{-1} and pH 8.0.

2.5. Extraction and Clean Up Method

QuEChERS method with slight modifications was used for the extraction and cleans up of cauliflower samples for estimation of indoxacarb residues [23,24]. Cauliflower curd samples were macerated in a blender (Blixer 6 V.V. by robot coupe, France) and a representative 15 g (±0.1 g) of the sample was transferred to 50 mL centrifuge tube. Added 30 mL of acetonitrile to each tube with the help of dispenser and homogenized @ 15,000 rpm for 2 - 3 min using a high speed homogenizer (High Speed Silent Crusher-Heidolph) to ensure that the solvent interacted well with entire sample. Mixed 5 - 10 g NaCl to each tube and shook the tubes on rotaspin for 5 minutes followed by centrifugation for 3 min @ 2500 rpm. The acetonitrile layer was collected in another centrifuge tube containing 5 g activated anhydrous sodium sulfate and again shaken for 5 minute at 50 rpm on the rotaspin to remove the moisture completely. The samples were cleaned up by dispersive solid phase extraction method. An aliquot of 6mL was dispensed into 15 mL centrifuge tube containing 0.15 ± 0.01 g (PSA) primary secondary amine sorbent (to remove fatty acid among other components) and 0.9 ± 0.01 g anhydrous $MgSO_4$ (to reduce the remaining water in the extract) and 0.05 ± 0.01 g graphatised carbon. The tubes were vortexed for 3 minutes followed by centrifugation for 1 min @ 2500 rpm. From the upper layer of the prepared samples, 4mL of the extract were transferred into another 15 mL tube and were rotary evaporated at <35˚ to remove the acetonitrile completely. Finally, the volume was reconstituted to 2 - 5 mL with distilled acetone. Soil samples were processed by adding 10 mL of distilled water in 15 g of soil sample and rest of the procedure was same as per the method described above.

3. Instrumental Analysis

The cleaned up extracts were analysed by Gas liquid chromatograh (Perkin Elmer Clarus 500) equipped with an electron capture detector (ECD) ^{63}Ni operated at 310˚C. Chromatographic separation was carried out using a capillary column Elite 608 (50 m × 0.53 mm i.d, 1.5 µm film thicknesses) held at 290˚C with spilt ratio 1:10 was used for the estimation of indoxacarb residues. The carrier gas flow was 2 $mL \cdot min^{-1}$ of high purity nitrogen with makeup flow of 30 $mL \cdot min^{-1}$. The injector port and detector was held at 300˚C and 310˚C respectively. Under these operating conditions the retention time of indoxacarb was 6.20 minutes. Various concentrations of standard solutions varying from 0.01 to 0.20 $\mu l \cdot mL^{-1}$ were prepared and 1 µL of each was injected to prepare the standard curve.

The residues of indoxacarb on cauliflower curds were confirmed by gas chromatograph-mass spectrometer (GC-MS) in selective ion monitoring (SIM) mode. The gas chromatograph (Shimadzu-QP 2010) with auto injector, equipped with mass spectrometer and capillary column Rtx-5 Sil MS (30 m × 0.25 mm i.d. × 0.25 µm film thickness) was used to verify the results. The GC-MS operating conditions were: injector temperature 285˚C, oven initial temperature was 200˚C and held for 4.0 min, raised to 280˚C at a rate of $10^{\circ}C \cdot min^{-1}$ and held for 10 min, ion source temperature 200˚C, interface temperature was 290˚C. Helium was used as a carrier gas with a flow rate of 1.0 $mL \cdot min^{-1}$.

4. Results and Discussions

4.1. Efficiency of Analytical Method

Validation is an essential requirement to ensure quality and reliability of the results for all the analytical applications [25].The factors considered in the validation included recovery, precision (relative standard deviation), determination coefficient (R^2), linearity, limit of detection (LOD) and limit of quantification (LOQ) [26-29]. In order to evaluate the efficiency of extraction, cleanup and determinative steps the analytical method was standardized by processing spiked samples, before taking up the analysis of the test samples. The experiment was performed by spiking the cauliflower and soil samples with the pesticide being studied. The recoveries were found to be consistent and more than 80% at different concentration (0.01, 0.05, 0.1, 0.2 $mg \cdot kg^{-1}$) with relative standard deviation RSD below 15% confirmed a good repeatability of the method shown in **Table 1.**

Table 1. Recovery studies and RSD values obtained for indoxacarb in cauliflower and soil at different spiking levels.

Substrates	Level of spiking ($mg \cdot kg^{-1}$)	[a]Recovery (%)	[*]RSD (%)
Cauliflower	0.20	94	6
	0.10	90	2
	0.05	96	1
	0.01	90	2
Soil	0.20	91	3
	0.50	94	2
	0.05	91	5
	0.01	87	1

[a]Each value is mean of four replicate determinations; [*]Relative standard deviation.

Further, linearity of the calibration curves was studied using pesticide standard solution at concentration ranging between 0.01 - 2 µg·mL^{-1} in GC-ECD detector. The response function was established to be linear. The calibration curve that constructed followed linear relationships with good correlation coefficients ($R^2 > 0.99$) with the equation of analytical graph was y = 3E + 06x – 7417 shown in **Figure 2**.

The LOQ of indoxacarb was found to be 0.01 mg·kg^{-1}, and no substrate interferences were observed at this detection limit as evidenced by control samples analysis shown in **Figure 3**. The limit of detection was determined as the concentration having a peak area three times higher in relation to the noise of the base line at the retention time of the peak of interest. The LOD was calculated to be 0.003 mg·kg^{-1}. Residues were estimated by comparison of peak height/peak area of the standards with that of the unknown or spiked samples run under identical conditions. The average data on indoxacarb residues were subjected to statistical analysis [30].

4.2. Residues of Indoxacarb in Cauliflower and Soil

The average initial deposits of indoxacarb on cauliflower were found to be 0.23 and 0.45 mg·kg^{-1} following the last application of indoxacarb 14.5 SC @ 52.2 and 104.4 g a.i. ha^{-1} shown in **Table 2**. Residues of indoxacarb

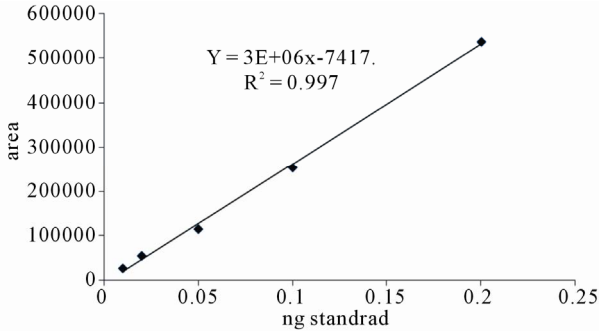

Figure 2. Linearity calibration curve for indoxacarb at concentration of 0.01 to 2 ng.

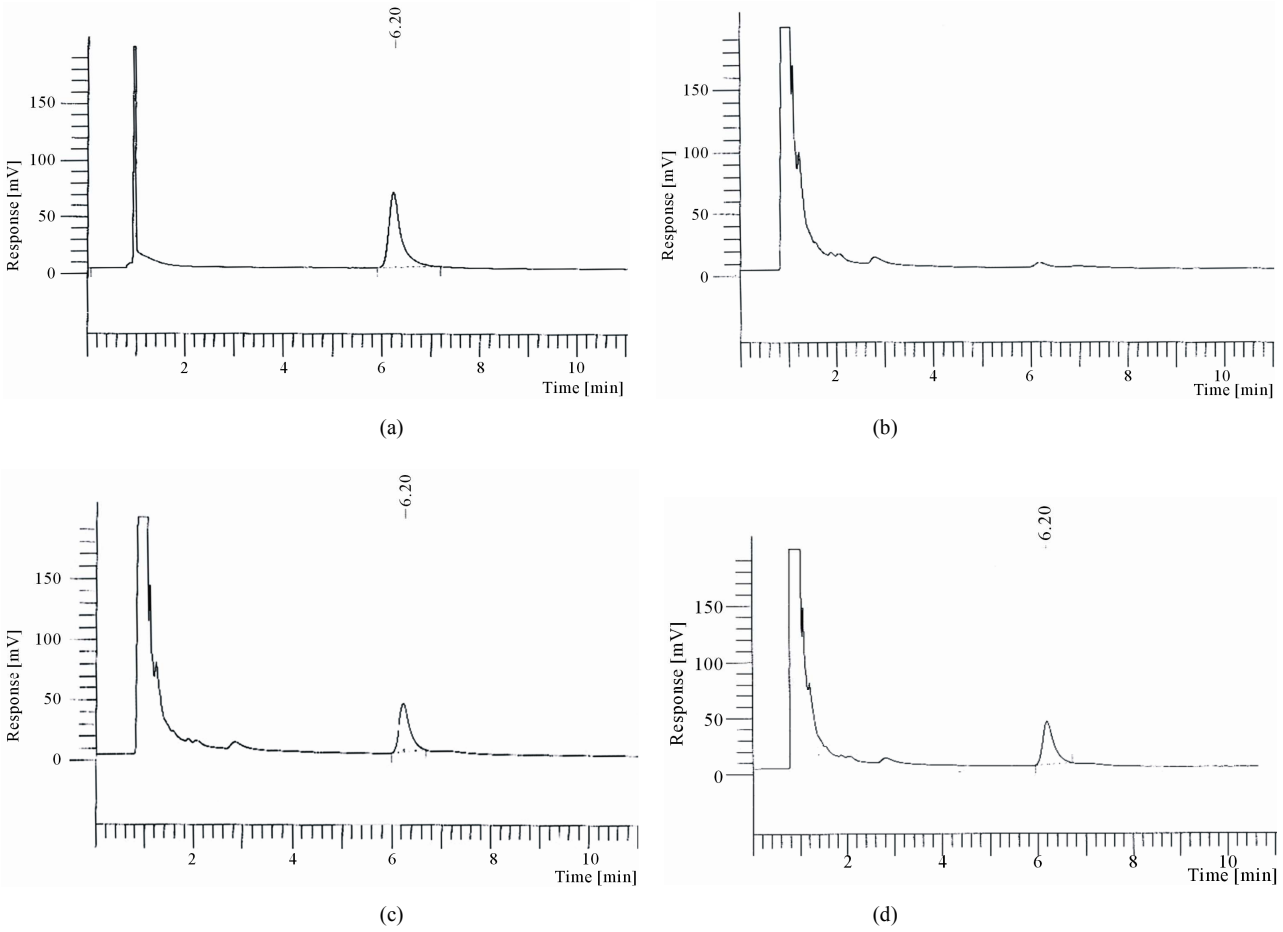

Figure 3. GC Chromatograms (a) Indoxacarb 0.02 ng standard; (b) Untreated cauliflower sample; (c) Spiked cauliflower sample; (d) Field treated cauliflower sample.

Table 2. Mean and range of indoxacarb residues (mg·kg^{-1}) in cauliflower and soil at different time interval after the application indoxacarb @ 52.2 and 104.4 g a.i. ha^{-1}.

Time after application (Days)	Indoxacarb @ 52.2 g a.i. ha^{-1}				Indoxacarb @ 104.4 g a.i. ha^{-1}			
	R_1	R_2	R_3	Mean ± SD	R_1	R_2	R_3	Mean ± SD
0 day (1hr after application)	0.26	0.20	0.21	0.23 ± 0.03	0.43	0.46	0.46	0.45 ± 0.017
1	0.15	0.13	0.15	0.14 ± 0.01 (39.14)*	0.24	0.23	0.22	0.23 ± 0.01 (44.44)*
3	0.05	0.06	0.05	0.05 ± 0.002 (78.26)*	0.10	0.14	0.19	0.14 ± 0.04 (68.88)*
5	0.01	0.01	0.02	0.01 ± 0.001 (95.65)*	0.03	0.04	0.03	0.03 ± 0.002 (93.33)*
7	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
10	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
15	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Soil sample after 15 days	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
#$T_{1/2}$ days	1.12				1.31			

()* Per cent dissipation after spraying; BDL < 0.01 mg·kg^{-1}; #$T_{1/2}$= Half-life time.

dissipated more than 78 and 68 percent after 3 days of the last application at single and double the dosages respectively. Sinha *et al.* [31] reported initial deposits of indoxacarb residues of 0.11 and 0.21 mg·kg^{-1} on brinjal following the application @ 70 and 140 g a.i. ha^{-1}, whereas, Gupta *et al.* [32] reported initial deposits of 0.26 and 0.67 mg·kg^{-1} on okra at the same dosages, respectively. Though the dose used in case of brinjal is higher than the dose used in cauliflower but the initial deposits of indoxacarb residues in brinjal were low because of the nature of substrate.

The results of dissipation of indoxacarb in cauliflower are shown in **Figure 4**. The residues of indoxacarb in cauliflower dissipated below its LOQ of 0.01 mg·kg^{-1} after 7 days with half-life periods observed to be 1.12 and 1.31 days, respectively, at single and double the dosages. The results were in agreement with Gupta *et al.* [32] who reported the half-life of 1.6 and 2.3 days on okra following application of indoxacarb @ 70 and 140 g a.i. ha^{-1}, respectively. However, indoxacarb residues were below the determination limit of 0.01 mg·kg^{-1} in the soil samples collected at harvest.

The residues of indoxacarb on cauliflower curds were confirmed by gas chromatograph-mass spectrometer (GC-MS) in selective ion monitoring (SIM) mode with low concentration. The fragmentation of indoxacarb produced selective m/z ions of 249, 293, 496, 529 and base peak at 529 shown in **Figure 5**. The treated samples also showed the presence of these ions which confirmed the presence of indoxacarb.

4.3. Risk Assessment

The maximum residue limit (MRL) of indoxacarb on cauliflower has been prescribed as 0.2 mg·kg^{-1} by Codex [33]. The statistical analysis revealed that the residues of indoxacarb on cauliflower dissipated below the MRL in 2.2 days. MRL for Indoxacarb under Indian condition is not available to make an assessment of the results obtained. Therefore, a risk assessment of indoxacarb residues on cauliflower was made on the basis of its total intake through consumption of cauliflower and comparing it to its acceptable daily intake (ADI). ADI of indoxacarb is 0.01 mg·kg^{-1} body weight·day^{-1}. Maximum Per missible Intake (MPI) was obtained by multiplying the ADI with the average weight (55 kg) of an Indian person (Mukherjee and Gopal, 2000). MPI was calculated to be 550 µg·person^{-1}·day^{-1}. Theoretical Maximum Residues Contribution (TMRC) has been calculated at considering recommended consumption of cauliflower as 80 g in Indian context [34]. The TMRC values were derived through maximum residue level observed from recommended the double the recommended dosages, respectively, and were observed to be 20.8 and 36.8 µg, respectively shown in **Table 3**. Both these values are signifi

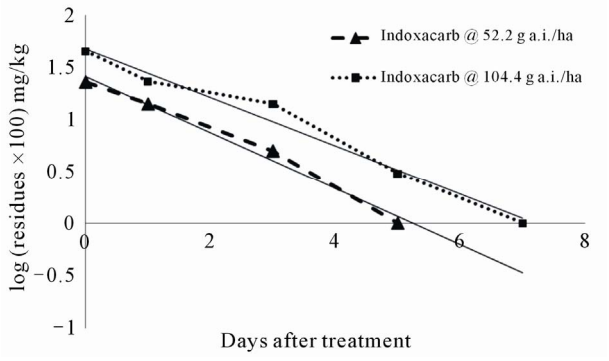

Figure 4. Semi logarithm graph of indoxacarb showing dissipation kinetics in cauliflower with Regression equation y = –0.269x + 1.408 (single dose) and y = –0.232x + 1.673 (double dose).

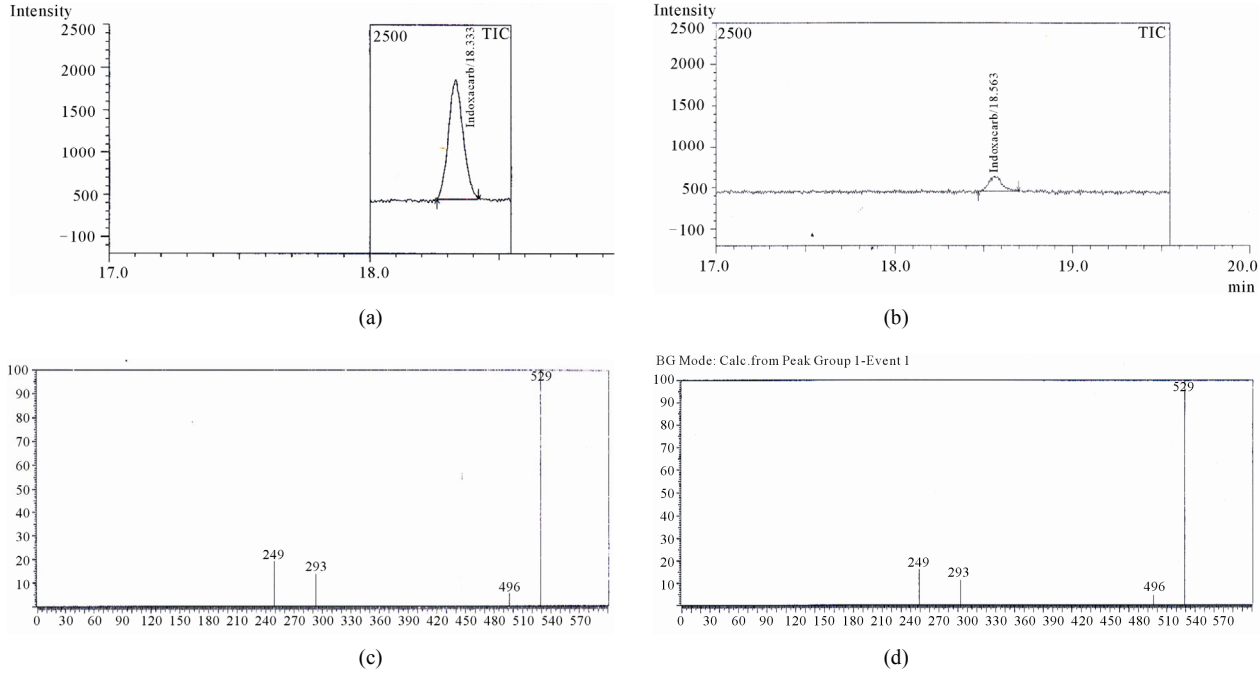

Figure 5. GC-MS Chromatograms (a) Standard indoxacarb; (b) Field treated caulifower sample; (c) Mass spectra of standard indoxacarb; (d) Mass spectra of indoxacarb field treated cauliflower sample.

Table 3. Theoretical maximum residue contribution (TMRC) in cauliflower curds.

Days	Maximum residues in cauliflower curds (mg·kg^{-1}) in T_1	TMRC µg·person^{-1}·day^{-1}	Maximum residues in cauliflower curds (mg·kg^{-1}) in T_2	TMRC µg·person^{-1}·day^{-1}
0	0.26	20.8	0.46	36.8
1	0.15	12.0	0.25	20
3	0.06	4.8	0.19	15.2
5	0.02	1.6	0.03	2.4
7	BDL	BDL	BDL	BDL
10	-	-	-	-
15	-	-	-	-

BDL Below determination limit (<0.01mg·kg^{-1}); T_1 Indoxacarb@ 52.2 g a.i. ha^{-1}; T_2 Indoxacarb@ 104.4g a.i. ha^{-1}.

cantly lower as compared to MPI. So, the dietary exposure to indoxacarb is within health standard with in safety zone and no health hazard is expected.

5. Conclusions

An analytical method for estimation of indoxacarb residues was standardised and validated. The residues of indoxacarb dissipated below its LOQ of 0.01 mg·kg^{-1} after 7 days with half-life periods of 1.12 and 1.31 day, respectively, at single and double the dosages. The statistical analysis revealed that the residues of indoxacarb dissipated below its prescribed MRL of 0.2 mg·kg^{-1} in 2.2 days. The TMRC values of indoxacarb were below its acceptable daily intake (ADI) at both the application rates when calculated from maximum residues levels observed on 0-day. Hence the use of indoxacarb is safe from crop protection point of view and residues in food are unlikely to pose any undue hazard to the consumers.

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