Journal of Advanced Research in Food Science and Nutrition
Volume 6, Issue 2 - 2023, Pg. No. 1-25
Peer Reviewed Journal

Research Article

Method Development and Validation of Residual Pesticides in Cumin

Geeta Mathur[1], Priyanka Sharma[2], Nidhi K[3], Manjeet A[4], R. K. Khandal[5]

[1]Research Scholar, Department of Basic Sciences, Dr. K.N. Modi University, Newai, Rajasthan, India.
[2]Research Scholar, Department of Basic Sciences, Dr. K.N. Modi University, Newai, Rajasthan, India.
[3] Quality Assurance Manager, CFRA, NIFTEM, Kundli, Sonepat, Haryana, India.
[4]Professor & Head of Department, Dept. of Basic and Applied Sciences & CFRA, NIFTEM, Kundli, Sonepat, Haryana, India
[5]Professor, Chief Mentor, Dr. K.N. Modi University, Newai, Rajasthan, India.

I N F O

Corresponding Author:
R. K. Khandal, Dr. K.N. Modi University, Newai, Rajasthan, India.
E-mail Id:
profrakeshkhandal@gmail.com
Orcid Id:
https://orcid.org/0000-0003-3121-9454
How to cite this article:
Mathur G, Sharma P, Nidhi K et al. Method Development and Validation of Residual Pesticides in Cumin. *J Adv Res Food Sci Nutr* 2023; 6(2): 1-25.

Date of Submission: 2023-08-17
Date of Acceptance: 2023-09-18

A B S T R A C T

India is the largest exporter of Spices in the world. Recent reports suggest that importers want the supplies to be strictly meeting the MRL limits of pesticide residues. Cumin seeds are under scanner by the largest importer China, EU and others for presence of pesticide residues. In order to ensure that the exports do not fall down, Spices Board of India issued an advisory to the stakeholders to monitor pesticide residue levels in all the lots and ensure that they meet the standards set by the regulators of importing countries. These demands for having simple, accurate and reliable methods of analysis of multi-residues in Cumin seeds. The present study deals with the development and validation of a multi-residue method for the simultaneous analysis of 26 pesticides in cumin seeds using gas chromatography mass spectrometry (GC-MS/MS). The aim of the study is to develop a reliable analytical method, which is adoptable by laboratories in India. The method involves the extraction of pesticide residues in acidic acetonitrile followed by analysis using QuEChERS technique with necessary modifications. Validated the method following the SANTE guidelines at the spike level of 5 ng/g and 10 ng/g for 26 pesticides. The method satisfies all the criteria of method validation including system suitability, linearity, and limit of quantification (LOQ), accuracy and precision as per the guidelines of SANTE /12682/2019. By using this method, it would be possible to quantify pesticide residues at as low as 10 ng/g level for all 26 pesticides in Cumin seeds.

Keywords: Spices, Cumin Seeds, Method Development, Method Validation, Pesticide Residues, Quechers

Introduction

Pesticides are agrochemicals vital for crop protection from pests, rodents, insects, fungus, weeds etc. In modern day agriculture, farmers have to use them at different stages of crops. The rising demand for food on one hand and shrinking area of arable land on the other hand presents a huge challenge for farmers. It is imminent to use different types of agrochemicals for the purpose of: a) controlling or repelling pests, b) eliminating fungus, c) destroying weeds, d) delayed ripening etc. because all of them are responsible for causing harm to crops during production, processing, handling, storage etc. of food.[1] Based on their chemical structure, functional group, mode of action, targeted organism etc., agrochemicals are classified in different categories. According to their functional groups, they are classified as organochlorine, organophosphates, carbamates, pyrethroids etc. Considering their applications, they are classified as pesticides, insecticides, rodenticides, fungicides, weedicides etc. Use of these agrochemicals results in increased crop yield as the former protect crops.[2,3]However, this presents the challenge of food safety because of the residues of pesticides left behind with the crop due to the kinetics of degradation of pesticides. It is not possible to have pesticides that degrade completely during the crop period and hence, traces of pesticides would always remain with the harvested crop. In recent times, presence of pesticide residues has become the biggest cause of concern for regulators across the world. The quality of food is determined by the level of residues of pesticides etc. present in them.[4] Depending upon the chemical dossier as well as biological dossier of every pesticide, regulators fix the maximum residue levels (MRLs) allowed in different foods. Food safety, now a days is synonym of food quality affecting the global food trade as MRL value of residues of pesticides etc. is the key parameter used to declare any food fit for human consumption. Often, there are reports of rejection of imports due to residue pesticides.[5] This causes disputes between the countries importing and exporting; importing countries reject citing the reasons of food safety whereas exporters contest terming the rejections as the practices of trade barrier. In order to avoid disputes, exporters try to comply with the food standards by monitoring the lots for residue levels using sensitive, robust, quick and reproducible methods.[6,7]

In the world, spices are largely produced, consumed and exported in India and in fact India is known as the land of spices. Amongst the spices, cumin seeds are the most important as they are used in almost all types of food preparations across the world. Cumin seeds are popular because of the presence of abundant essential oil content, their unique flavor, strong aroma and various functional properties beneficial for health and well-being. The quantities of Cumin seeds produced during five years period from 2017-2018 onwards ranged from ~6.9 lakhs tons /year to ~9.2 lakhs tons/year (Spice Board of India) showing poor rate of growth.[8,9] Before proceeding further on this subject, it is better to have an understanding of the production, market trends and needs of the stakeholders of exports of cumin seeds in India. Gujarat is the largest producer state of India followed closely by Rajasthan. The contribution by these two states accounts for more than 95% of the total quantity produced because of the fact that the climatic conditions of dry and scarce rainfall suit cumin farming the most.[10] Agencies involved in the export of Cumin seeds keep the farmers regularly updated about the need for organic Cumin for exports in future. Generally, the per hectare consumption of pesticides for cumin farming is much lower than what is in use for other crops. In spite of this, there are incidents of pesticide residues reported in exported lots. That is why, Spice board of India had to issue advisory on this matter. Rising global demand of cumin seeds due to the increased awareness about use of herbal and spices products for immunity-building, especially after covid-19 pandemic, presents an opportunity for farmers.[11] However, regulatory requirements asking for pesticide-residue-free-cumin is a challenge, looking at the scenario that the production is largely from two states; any drop in production in either Gujarat or Rajasthan will result a drop in quantities available for export.

Of the two types of seeds (small and big size) exported from India, the seeds of small size are more popular due to their superior quality and aroma. The major constituents present in Cumin seeds are an aldehyde (4-isopropylbenzaldehyde) and terpenes (alpha pinene and cis-B-farnescene). Even though the functional properties are due to the presence of these inherent constituents, the regulators would check more for the presence of pesticide residues rather than functional constituents. That is why the exporters analyze each lot for pesticide residues.[12] The most challenging task here is to have a robust and reliable method that can analyze various residues present in the spices. While developing the suitable method, one has to take care of the two critical aspects: a) complex and interfering matrix and b) multi-residues having different chemistry and polarities. The present study deals with method development and validation for multi-residues of pesticides in cumin seeds as per the requirements of the industry and envisaging the above-mentioned two challenges. In 2022-2023, India exported ~186,509 tons (Spice board of India).[13,14] This will grow with time, provided there is increase in production and improvement in quality. There are challenges, in raising the quantities for exports, due to the presence of residues. Thus, in order to be able to benefit from the rising demand, it is imminent to monitor quality of lots by residue analysis using reliable and accurate methods. As evident from

recent publications (Manirakiza et al., 2000; Donia et al., 2001; Ambrus et al., 2010; Jadhav et al., 2017; Shabeer et al., 2018; Goon et al., 2018; Goon et al., 2019; Styliani E. Romniou et al., 2022; Ramesh Babu Natarajan et al., 2022) development of methods, free from matrix interferences is a priority.

As per a recent article.[15] based on the data from the Ministry of Commerce and Industry, Government of India, the total exports of cumin seeds during 2020-2021 was ~ USD 185 million. The value (million USD) of cumin seeds exported to top ten countries were: i) China, ~32; ii) Bangladesh, ~25; iii) Vietnam, ~18; iv) Turkey, ~15, v) USA, ~15; vi) UAE, ~13; vii) Iran; ~12; viii) Malaysia, ~11; ix) Egypt, ~9; x) Saudi Arabia, ~8. While China imported ~22% of the cumin seeds exported, the share of exports to USA was also substantial ~8% of the total. Even though the demand is rising, the trends suggests that importers would prefer looking for organic cumin seeds. In future, therefore, strict monitoring of residues of pesticides would be imminent. Several extraction and clean-up techniques as reported by Shabeer et al., 2018; Rutkowska et al., 2018; Amate et al., 2010; Kandaswamy et al., 2021; Goon et al., 2019; Ramesh Babu Natarajan, 2022 were useful in developing methods of multi-residue analysis in spices. These are: a) dispersive solid phase extraction (d-SPE) and clean-up, b) freezing out fatty matrix co-extractives, c) dilution of final extract before analysis, d) solvent exchange before injection and e) hydrophilic-lipophilic-balance (HLB) cartridge SPE cleanup.[16,17] This study deals with development and validation of a method for multi-residue analysis of cumin spice with modifications in QuEChERS technique. Since, the pH of acetonitrile is critical parameter that affects the performance of extraction procedure therefore added 1% acetic acid to acetonitrile in order to improve the extraction of polar (e.g., organophosphate) pesticides. The polar matrix interferences like free fatty acids, sugars and some ionic lipids were reduced by the addition of Primary Secondary Amine (PSA), Dispersive Sorbent Material C18 improved pesticide detection and helped in removal of non-polar interferences, Graphitized Carbon Black (GCB) was added to minimize the color of the matrix. Developed and validated an easily adoptable, simple, sensitive and robust method for multi-residue (of 26 pesticides) analysis in cumin seeds by using GC-MS/MS.[18,19,20,21]

Materials and Methods

Chemicals and materials

The certified reference materials of 26 pesticides and triphenyl phosphate used as internal standard of purity greater than 98% were procured from Sigma-Aldrich (St. Louis, MO USA) and Dr. Ehrenstorfer GmbH (Augsburg, Germany). All the other reagents such as acetonitrile and ethyl acetate procured from local sources were of MS grade. Magnesium sulfate ($MgSO_4$), sodium chloride (NaCl), trisodium citrate dihydrate, disodium hydrogen citrate sesquihydrate, primary Secondary Amine (PSA), dispersive Sorbent Material C18 and graphitized Carbon Black (GCB) were sourced from Agilent Technologies, USA. Water of HPLC grade was used for sample preparation etc. Acetic acid used was AR grade.

Preparation of pesticide standard solutions

Accurately weighed ~10 mg of each of the 26 pesticides (under study) in separate (for each of the pesticides) 10 ml volumetric flasks, dissolved them in ethyl acetate and made up their volume with ethyl acetate. These solutions were referred as stock solutions of the concentration of 1000 µg/g for each pesticide. On the basis of the concentration of stock solution of different pesticides, standard solutions of pesticide mix were prepared. For this purpose, different volumes of stock solutions of individual pesticide aliquots from each stock solution made above were mixed together to obtain a pesticide mix having same concentration of each of the 26 pesticides. By diluting this pesticide mix with ethyl acetate, a series of standard solutions of concentrations; 100 µg/g, 10 µg/g, 1 µg/g, 100 ng/g referred as primary standard solutions were prepared. For method development, the primary standard solutions of concentrations of 1 µg/g and 100 ng/g were used in the study to prepare matrix matched calibration standards using ethyl acetate as the solvent.

Preparation of internal standard (IS)

Accurately weighed ~10 mg of triphenyl phosphate (IS) in a 10 ml volumetric flask, dissolved in ethyl acetate and made up the volume with ethyl acetate. This solution was referred as stock solution having the concentration of 1000 µg/g. Aliquots of different dilutions (of concentrations of 100 µg/g, 10 µg/g and 1 µg/g) from the stock solution were made using ethyl acetate as a solvent. All these solutions were used as the primary internal standard solutions.

Preparation of matrix matched calibration solutions

Accurately weighed 1.0 g (±0.05 g) of matrix blank cumin powder in ten separate polypropylene centrifuge tubes each of 50 ml. Added 10 mL HPLC grade water, vortexed the contents for one minute and kept it on shelf for 30 minutes. After that added 10 mL of acetonitrile solution containing 1% by volume of acetic acid and vortexed for one minute followed by addition of 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate sesquihydrate. Mixed the contents rigorously by shaking to prevent formation of lumps. Vortexed the contents for one minute followed by centrifuging them for 5 min at 5000 rpm and at cold temperature (4ºC±2 ºC). From this, transferred 6.0 mL of supernatant layer into a 15 mL centrifuge tube containing 900 mg of $MgSO_4$, 300

mg of PSA, 300 mg C_{18} and 45 mg GCB. Vortexed them for 1 min and centrifuged at 10,000 rpm for 10 mins at cold temperature (4°C±2 ºC). From this, transferred 3.0 mL of the supernatant into a separate centrifuge tube and evaporated the solvent off, by purging with gentle stream of nitrogen while maintaining the temperature at 35°C±2°C. Reconstituted the remnants with 1 mL of ethyl acetate and filtered them through 0.22-micron PTFE membrane filter. Combined all 1 ml extract and this was referred as matrix blank after undergone the sample procedure. For making matrix match calibration standards, seven volumetric flasks were taken. To each flask, added required matrix blank and required aliquots as mentioned in table from the primary standard solutions of concentrations 100 ng/g and 1000 ng/g were added to obtain concentrations of 1.5 ng/g, 3 ng/g, 10 ng/g, 50 ng/g, 100 ng/g, 200 ng/g. Also, internal standard (triphenyl phosphate) of concentration 1µg/g was added to obtain concentrations of 50 ng/g in each centrifuge tube. Made up the volume to 1 ml with matrix blank as mentioned in Table-1.

Table 1.Preparation of matrix match linearity standards

Matrix match Linearity (ng/g)	Pesticide mix (mL)	Internal standard concentration (50 ng/g)	Matrix blank (mL)	Final volume (ml)
Matrix Blank	0	50 mL from 1 µg/g	950	1
1.5	15 mL from 100 ng/g	50 mL from 1 µg/g	935	1
3	30 mL from 100 ng/g	50 mL from 1 µg/g	920	1
10	100 mL from 100 ng/g	50 mL from 1 µg/g	850	1
50	50 mL from 1 µg/g	50 mL from 1 µg/g	900	1
100	100 mL from 1 µg/g	50 mL from 1 µg/g	850	1
200	200 mL from 1 µg/g	50 mL from 1 µg/g	750	1

Sample preparation

For sample preparation, the QuEChERS method was modified by making certain changes pertaining to reduction of the sample weight and to change the nature of the solvent (acetonitrile) by adding acetic acid. All such changes were affected after a series of experiments of recoveries by varying the sample size as well as the content of acetic acid added to acetonitrile. The modifications in QuEChERS method were made by arriving at the minimum possible sample size and minimum amount of acetic acid in acetonitrile. The sample preparation with modified QuEChERS method is as below.

Accurately weighed 1.0 g (±0.05 g) of cumin powder sample in a 50 mL polypropylene centrifuge tube. Added 10 mL of water and vortexed the contents for one minute and kept it on shelf for 30 minutes. After that added 10 mL of acetonitrile solution containing 1% by volume of acetic acid and vortexed them for one minute followed by addition of 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate sesquihydrate and mixed the contents rigorously by shaking to prevent formation of lumps. Vortexed the contents for one minute followed by centrifuging them for 5 min at 5000 rpm and at cold temperature (4ºC±2 ºC). From this, transferred 6.0 mL of supernatant layer into a 15 mL centrifuge tube containing 900 mg of $MgSO_4$, 300 mg of PSA, 300 mg C_{18} and 45 mg GCB. Vortexed them for 1 min and centrifuged at 10,000 rpm for 10 mins at cold temperature (4°C±2 ºC). From this, transferred 3.0 mL of the supernatant into a separate centrifuge tube and evaporated the solvent off, by purging with gentle stream of nitrogen while maintaining the temperature at 35°C±2°C. Reconstituted the remnants with 1 mL of ethyl acetate and filtered them through 0.22-micron PTFE membrane filter. Transferred the filtrate as the final extract (sample prepared for analysis of residues) into auto-sampler vials for analysis by GC-MS/MS.

Recovery Studies

For recovery studies, accurately weighed 1.0 g (±0.05 g) blank cumin powder samples in a 50 mL polypropylene centrifuge tube. Spiked the samples at concentration levels of 5 ng/g and 10 ng/g (Table-2). Both the concentrations were prepared in triplicates. Also, added internal standard (triphenyl phosphate) in all the centrifuge tubes including matrix blank at a concentration level of 50 ng/g (Table-2). Samples were kept for 15 minutes at ambient temperature prior to their use. Added 10 mL of acetonitrile solution containing 1% by volume of acetic acid and vortexed them for one minute followed by addition of 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogen citrate sesquihydrate and mixed the contents rigorously by shaking to prevent formation of lumps. Vortexed the contents for one minute followed by centrifuging them for 5 minutes at 5000 rpm and at cold temperature (4ºC±2 ºC). From this, transferred 6.0 mL of supernatant layer into a 15 mL centrifuge tube containing 900 mg of $MgSO_4$, 300 mg of PSA, 300 mg C_{18} and 45 mg GCB. Vortexed for 1 minute and centrifuged at 10,000 rpm for 10 minutes at

cold temperature (4°C±2 ºC). From this, transferred 3.0 mL of the supernatant into a glass tube and evaporated the solvent off, by purging with gentle stream of nitrogen while maintaining the temperature at 35°C±2°C. Reconstituted the remnants with 1 mL of ethyl acetate and filtered them through 0.22-micron PTFE membrane filter. Transferred the filtrate as the final extract (sample prepared for analysis of residues) into auto-sampler vials for analysis by GC-MS/MS. The recoveries were calculated using the calibration curves constructed using spiked samples as described in matrix matched calibration curves.

Table 2.Preparation of recovery samples and matrix blank

Recovery Concentration (ng/g)	Pesticide mix (mL)	Internal standard concentration (50 ng/g)	Sample weight (g)	Dilution (ml)	Pipetted out (ml)	Final volume (ml)
Matrix Blank	0	50 mL from 1 µg/g	1.0 g (±0.05 g)	10	3	1
5	50 mL from 100 ng/g	50 mL from 1 µg/g	1.0 g (±0.05 g)	10	3	1
10	100 mL from 100 ng/g	50 mL from 1 µg/g	1.0 g (±0.05 g)	10	3	1

Method Optimization and Selection of Method Suitable for the Purpose

Several trials by varying the conditions of the GC-MS/MS were undertaken to determine the most optimum parameters for method development of the pesticide residues under study. After analyzing the standard solutions for qualitative as well as quantitative determination of pesticides individually in the pesticide mix, under varying conditions of GC-MS/MS, the optimum conditions suitable for the method development were chosen as described below.

Instruments

A GC-MS/MS system (7000 Network GC system chromatograph with a triple quadrupole, QQQ detector, 7890 D of Agilent technologies, Wilmington, USA. The equipment was attached with an autosampler (Agilent technologies, Wilmington, USA). For separation, two capillary columns (HP-5 MS) each of length 15 m, internal diameter 0.25 mm and film thickness 0.25µm were used.

Identification of Pesticides

Qualitative determination of pesticide residues in cumin was analyzed in GC-MS/MS through GC-MS Browser software Mass Hunter data Acquisition (Agilent, USA). The identification was done by comparing the quantifier ion peak and the qualifier ion peak for each pesticide. The ion ratios of two transitions of samples and matrix-matched standards (±30%) were compared. The standard deviation for retention time (±0.1 min) of each of the pesticides was also checked. This was done by retention time locking using chlorpyrifos methyl pesticide as the standard. This allowed the qualitative determination (identification) of each of the pesticides with clarity. The variation in retention time of each of the pesticides was found to be within the range of ±0.1 min by using this approach.

GC-MS/MS analysis

The GC-MS/MS was employed with helium as a carrier gas, with the constant flow rate of 1.0 ml/min. The oven temperature programed at 60°C for 1 min hold increasing to 120°C at the rate of 40°C/min and then increased to 310°C at the rate of 5°C/min and then holding to 280°C for 5 minutes. Injection port was adjusted at 280°C and split less injection was used. Temperature for transfer line was kept at 280°C, Ion source temperature was kept at 300°C, MS Quadrupole temperature was kept at 180°C. Nitrogen gas of purity (99.999%) was used as collision gas and collision gas flow was kept at 1.5 ml/min. Backflush was done during post-run for 5 min. Ionization mode used was electron impact ionization. Retention time locking was done with chlorpyrifos methyl standard and was locked at 18.11 minutes.

After acquisition of the total ion chromatogram for the mixed stock standard solution in scan mode, peaks were identified by their retention time and mass spectra. The most abundant ion that showed no evidence of chromatographic interference and had the highest signal to noise ratio was selected for quantification purpose.

Quantitative Determination of Pesticide Residues

The quantification of pesticide residues was done by extrapolating a matrix-matched calibration curve for each pesticide at six levels of concentration levels of 1.5 ng/g, 3.0 ng/g, 10.0 ng/g, 50.0 ng/g, 100.0 ng/g and 200.0 ng/g using the precursor ion peak area, which is usually the most abundant transition. The matrix-matched calibration curve was made by spiking samples with different volumes of a multi-standard solution of pesticides in ethyl acetate. The calculation of the curve equation was performed through QQQ Quantitative Analysis data processing software and

GC-MS/MS Browser software. The calibration graph can be described by the equation y = mx + c, where y is the peak area and x is the pesticide concentration. Based on the calibration curve the concentration of various analytes was ascertained.

Method development and validation

Having optimized the conditions of GC-MS/MS for quantitative analysis of individual solution of pesticide mix and the recovery studies the method was developed by adopting modified QuEChERS method as explained above, a method was developed to achieve the desired LOQ and LOD. The method would be adoptable only when it is validated. Thus, validation is a must for all analytes as it proves and provides data that the method is fit for use. At least five replicates are required in the LOQ of the method (the lowest spiked level), and one higher level. Therefore, for the experimental part of the validation what was required was one blank sample (solvents only), one non-spiked sample (matrix only), five spiked samples at LOQ, and matrix matched samples (for the calibration curve). It has to be underlined that, according to SANTE/12682/2019, LOQ is defined as the lowest spike level meeting the identification and method criteria for recovery and precision. This is a common strategy followed when in case of multiresidue methods, so as not to proceed in enormous mathematical calculations. For spices, the lowest MRL for pesticide residues set by the USA, EURL is, 0.010 mg/kg. Therefore, LOQ was set to 0.010 mg/kg or 10 ng/g. The validation of the developed method was carried out as per SANTE guide lines with different parameters like specificity, system suitability (area and retention time of lowest calibration standard), linearity ($R^2 \geq 0.98$ was defined as internal criterion), Limit of Detection (LOD), Limit of Quantification (LOQ), precision (repeatability) and accuracy (recovery).

Results

GC-MS/MS determinations

All the analyses were performed in MRM mode based on the use of one target and two qualifier ions. The quantitation was based on the peak area ratio of the targeted quantifier ion to that of internal standard. The results of the analysis of

Table 3.Results of Retention time, MRM and collision energy of all the 26 pesticides

S. No.	Compound Name	Retention time (min)	Precursor Ion	Product Ion	Collision energy
1	Dichlorvos	5.78	184.9	63	25
			184.9	93.0	10
			144.9	109.0	10
2	EPTC	6.77	189.1	128.0	5
			189.1	86.0	10
			189.1	100.0	15
3	Cadusafos	11.77	158.8	97	15
			158.8	131	5
			157.9	96.9	15
4	BHC-gamma (Lindane)	13.43	216.9	181.0	5
			181.0	145.0	15
			183.0	147.0	15
5	Dimethenamid	16.21	230.0	154.1	10
			202.9	126	20
			232.0	154.1	10
	Dimethenamid-P	16.21	229.9	154.0	10
			231.9	154.0	10
			202.9	154.0	10
6	Chlorpyrifos-methyl	16.59	285.9	92.9	20
			124.9	47.0	15
			285.9	207.7	15
7	Alachlor	17.02	188.1	160.2	10
			188.1	132.1	15
			160.0	145.2	10

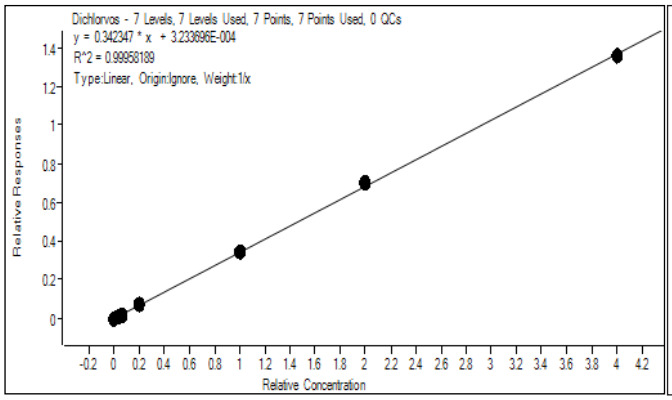
8	Metalaxyl	17.33	234.0	146.1	20
			234.0	174.1	10
			206.1	132.1	20
9	Malathion	18.82	172.9	99.0	15
			157.8	125.0	5
			157.8	47	25
10	Chlorpyrifos	19.25	196.9	169.0	15
			198.9	171.0	15
			313.8	257.8	15
11	Pendimethalin	21.01	251.8	162.2	10
			280.8	251.9	5
			161.9	161.1	10
12	Quinalphos	21.66	192.9	129	10
			298	155.9	10
			192.9	101.9	30
13	Procymidone	21.96	282.8	96	10
			96	53.1	15
			96	67.1	10
14	Profenofos	23.93	207.9	63.0	30
			338.8	187.8	30
			338.8	268.7	15
15	Ethion	26.03	230.9	175	10
			152.9	96.9	10
			124.9	96.9	0
16	DDE-o,p’	22.51	246.0	176.2	30
			248.0	176.2	30
			317.8	248.0	15
	DDE-p,p’	24.03	246.1	176.2	30
			317.8	246.0	15
			317.8	248	15
	DDD-o,p’	24.37	235.0	165.2	20
			235.0	200.2	10
			237	199.1	10
	DDD-p,p’	25.71	234.9	165.1	20
			234.9	199.1	15
			236.9	165.2	20
	DDT-o,p’	25.80	235.0	165.2	20
			199	163.1	35
			237.0	165.2	20
	DDT-p,p’	27.01	235.0	165.2	20
			235.0	199.2	15
			165.0	115.1	30

17	Trifloxystrobin	27.34	186	145.1	15
			116.0	89.0	15
			116.0	63.0	30
18	Piperonyl butoxide	27.94	176.1	103.1	25
			176.1	131.1	15
			176.1	117.1	20
19	Bifenthrin	28.89	181.2	165.2	25
			181.2	166.2	10
			166.2	165.2	20
20	Fenpropathrin	29.04	207.9	181.0	5
			181.1	152.1	25
			181.1	127	30
21	Fenazaquin	29.15	160.0	145.2	5
			160.0	117.1	20
			145.0	117.1	10
22	Pyriproxyfen	29.92	136.1	78.1	20
			136.1	96	15
			226.1	186.2	15
23	Cyhalothrin (lambda)	30.44	208.0	181.0	5
			181.1	127	30
			197.0	141.0	10
24	Cypermethrin I	32.80	162.9	127	5
			163.1	91	10
	Cypermethrin II	32.96	162.9	127	5
			163.1	91	10
	Cypermethrin III	33.09	162.9	127	5
			163.1	91	10
	Cypermethrin IV	33.15	162.9	127	5
			163.1	91	10
25	Etofenprox	33.28	163.0	135.1	10
			163.0	107.1	20
			135.0	107.0	10
26	Difenconazole I	35.26	322.8	264.8	15
			264.9	202	20
	Difenconazole II	35.42	322.8	264.8	15
			264.9	202	20

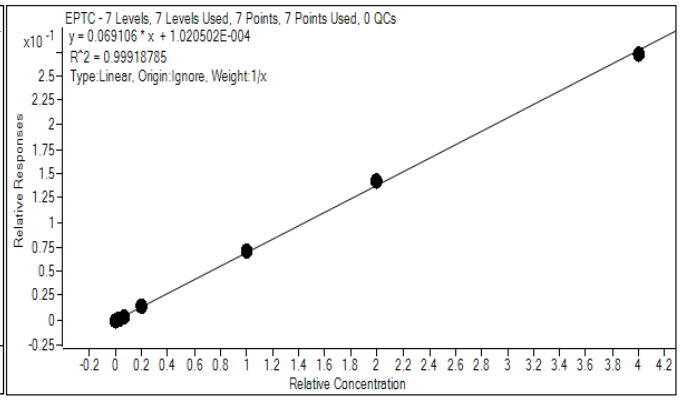
pesticides studied with their quantification and qualification ions used in MRM mode in this study are summarized below in Table 3.

The calibration curves prepared by plotting peak area and concentration of each pesticide in cumin samples. Six replicates for each concentration of pesticides (1.5 ng/g, 3 ng/g, 10 ng/g, 50 ng/g, 100 ng/g, 200 ng/g) were injected and analyzed. The calibration curves were prepared found linear, with correlation coefficient (r) ranging within an acceptable range of 0.995 to 1.0 for the different concentrations ranging from 1.5 ng/g to 200.0 ng/g. This calibration curve of each of the pesticides was used for quantification of respective pesticides. The results are very much in accordance with the standard guidelines

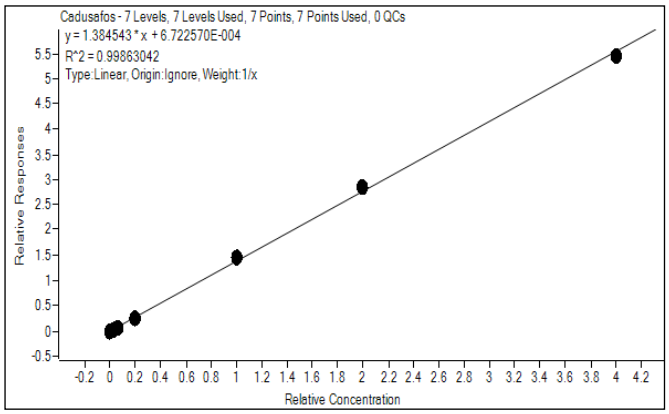
Dichlorvos



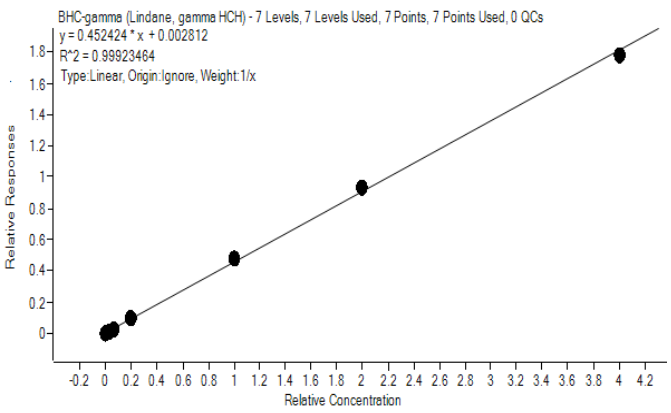
EPTC



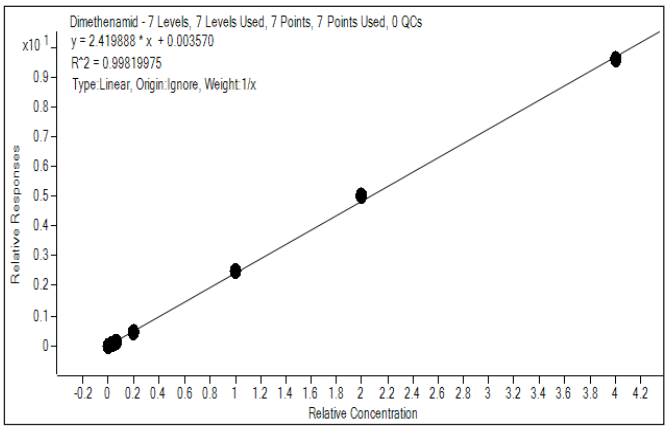
Cadusafos



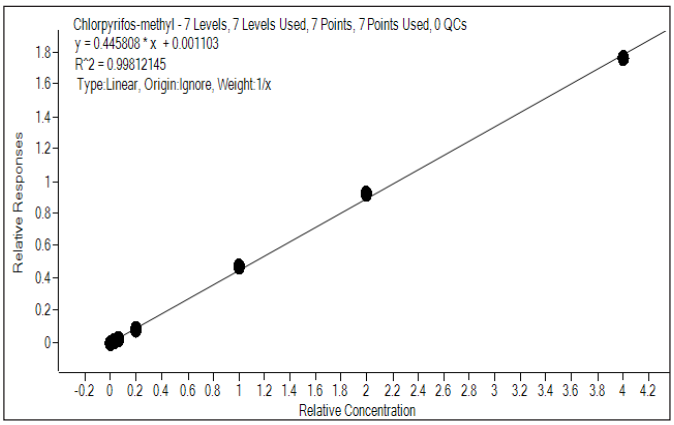
Lindane



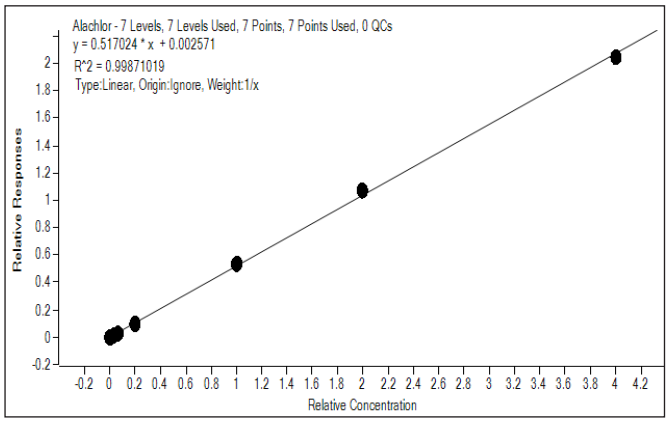
Dimethenamid



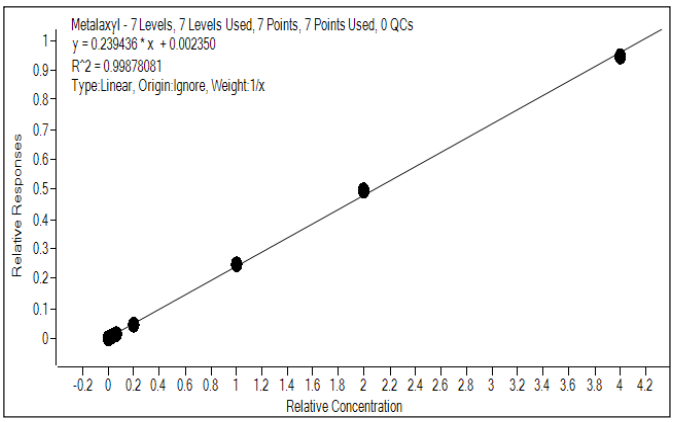
Chlorpyrifos methyl



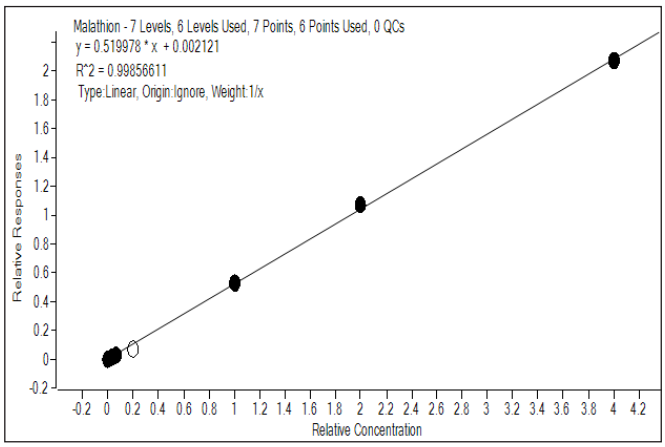
Alachlor



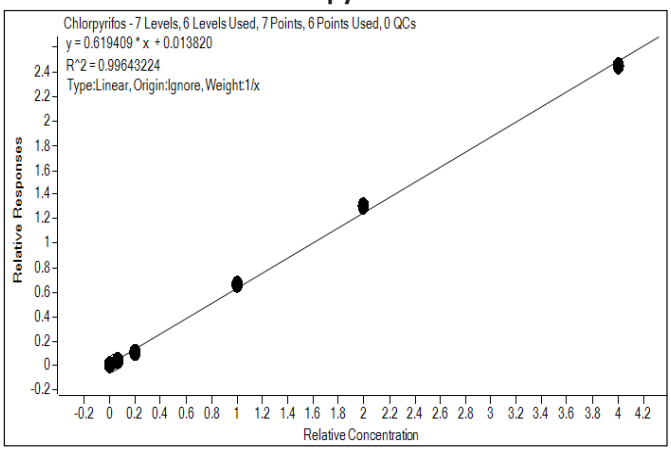
Metalaxyl



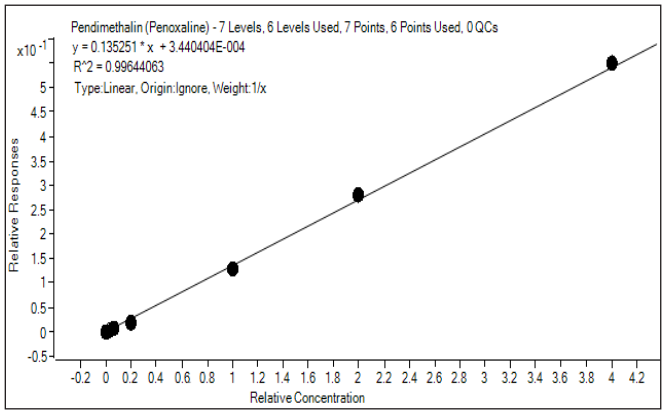
Malathion



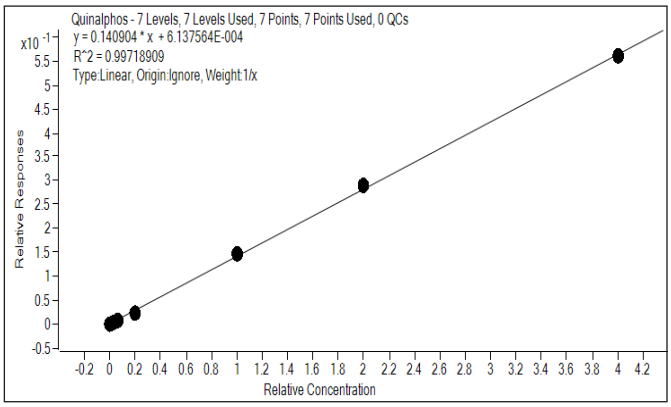
Chlorpyrifos



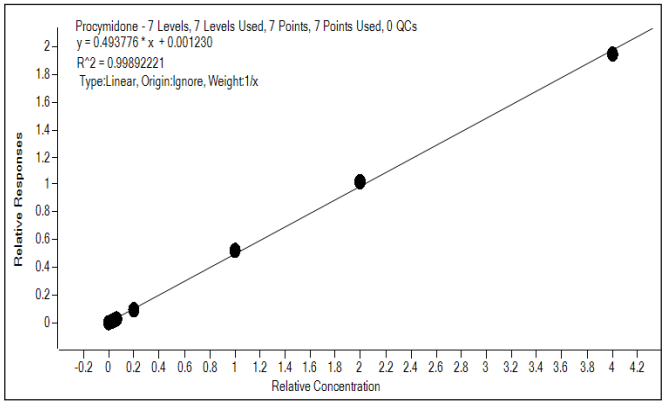
Pendimethlin



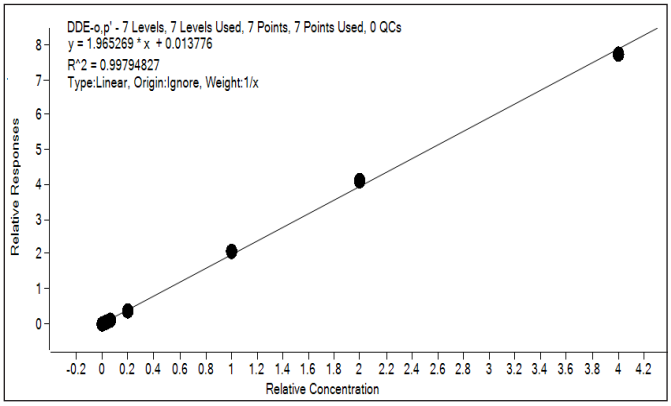
Quinalphos



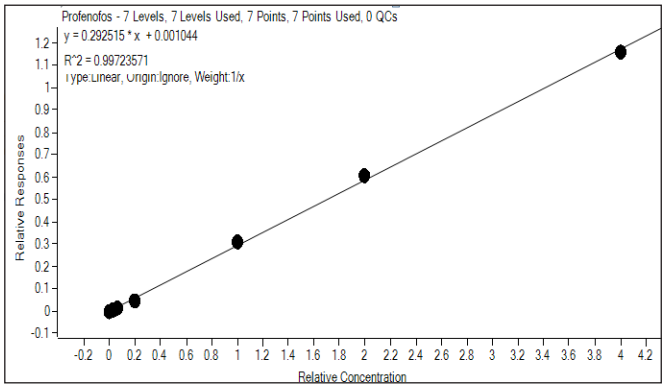
Procymidone



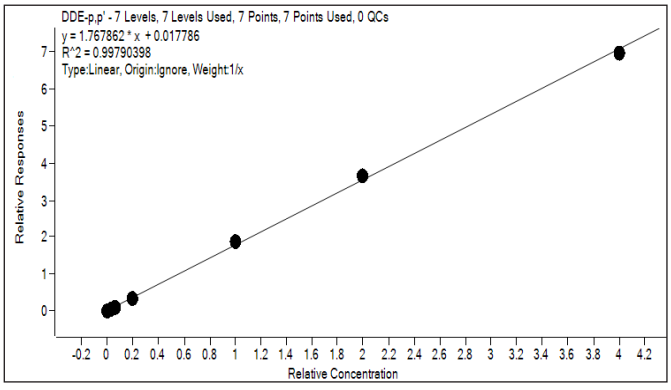
o,p-DDE



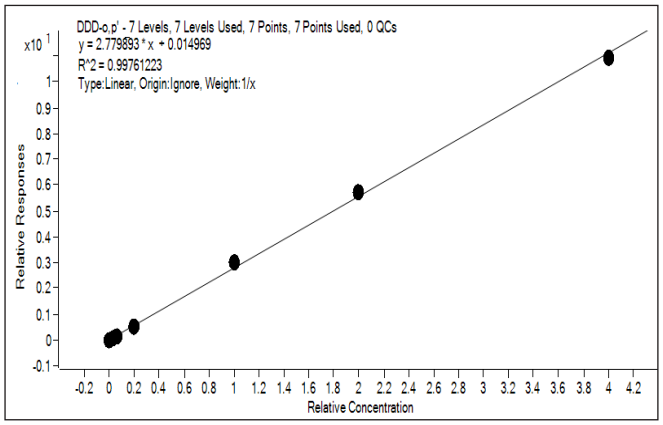
Profenofos



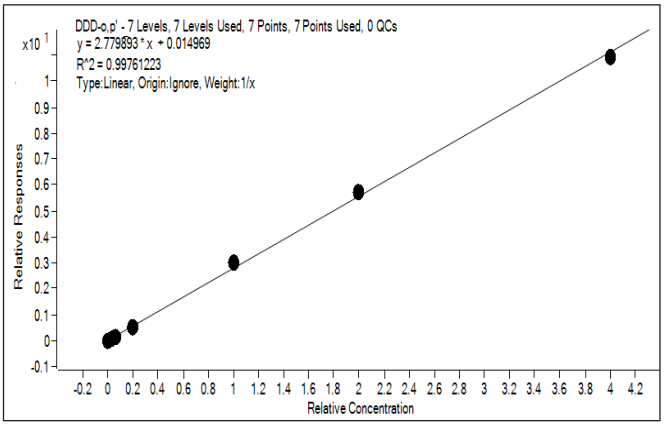
p,p-DDE



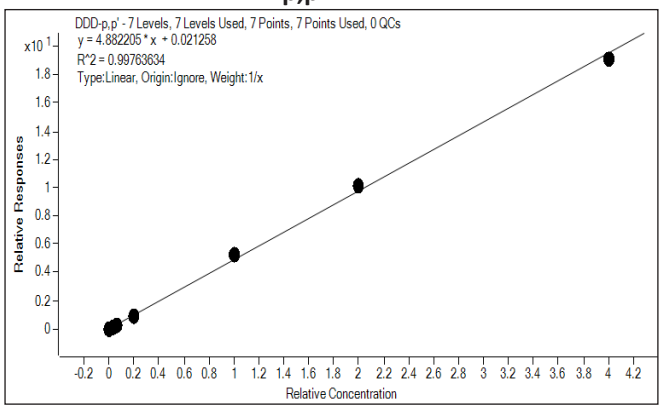
o,p-DDE



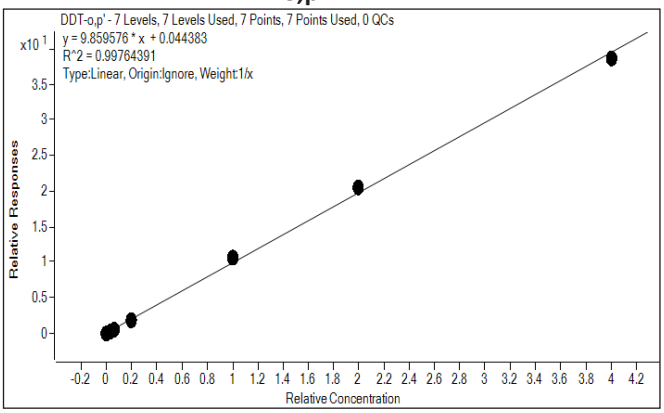
o,p-DDD



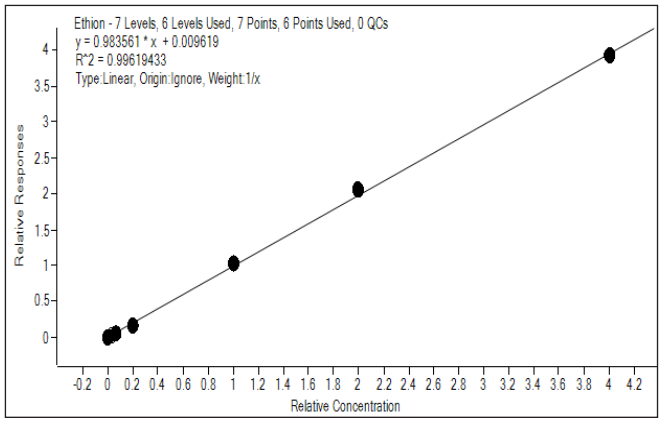
p,p-DDD



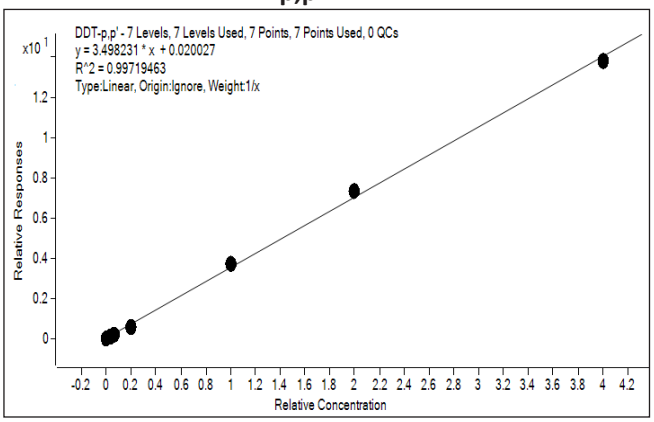
o,p-DDT



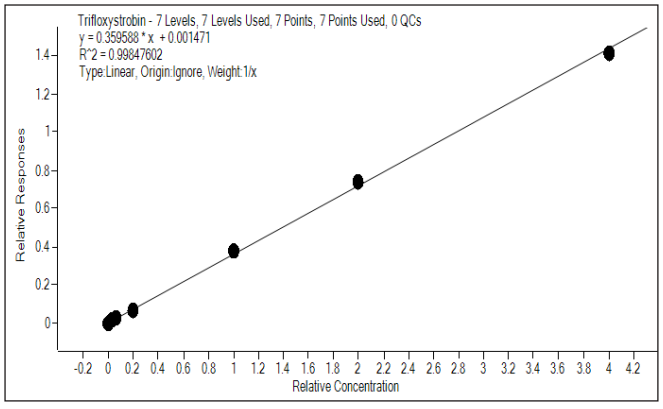
Ethion



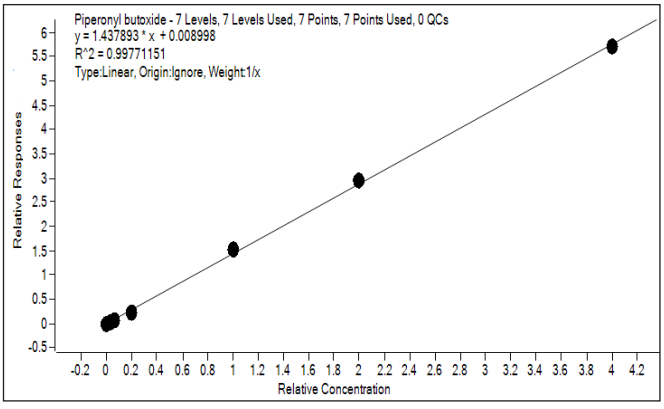
p,p-DDT



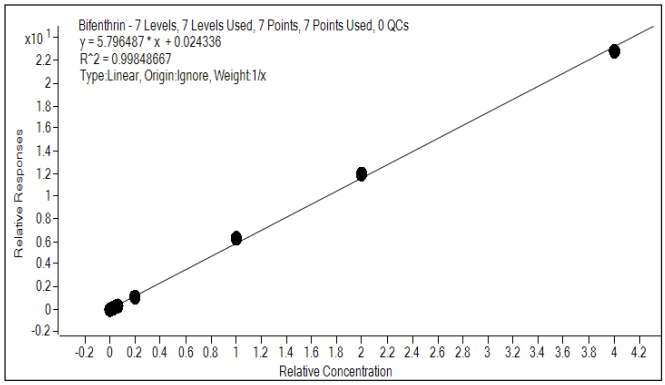
Trifloxystrobin



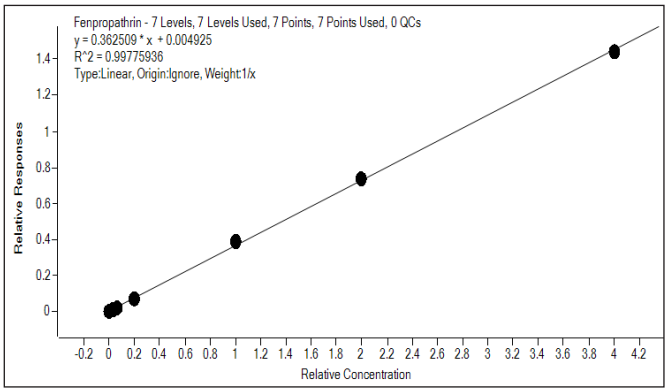
Piperonyl butoxide



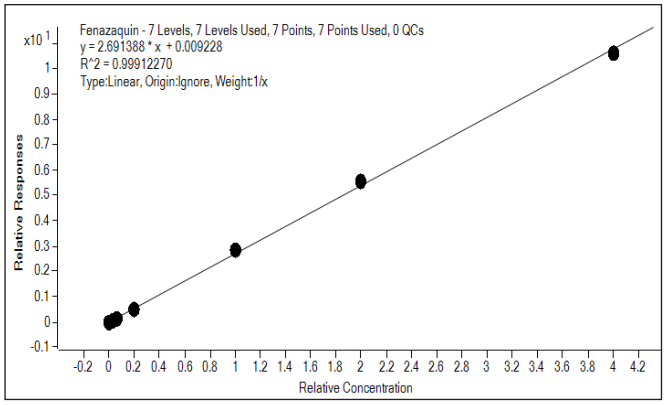
Bifenthrin



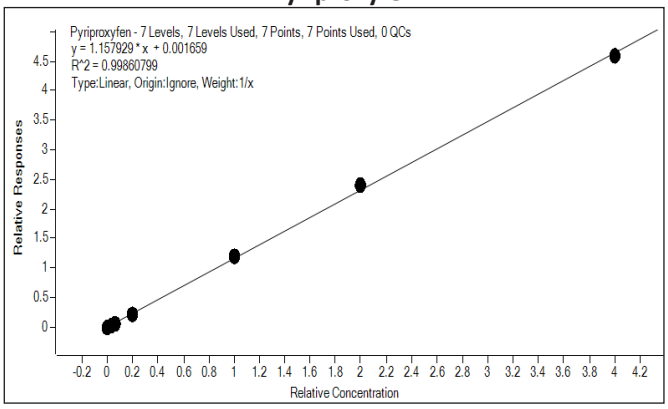
Fenpropathrin



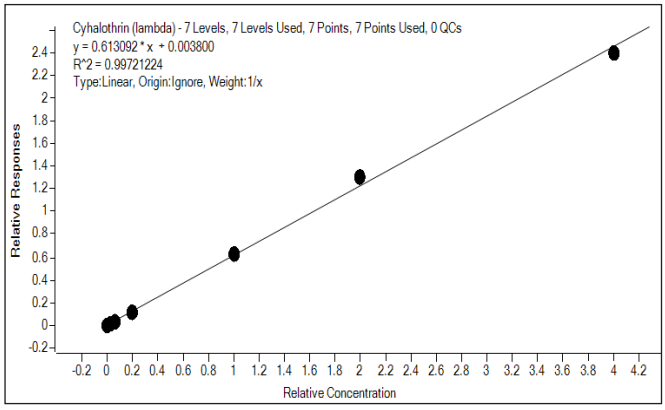
Fenazaquin



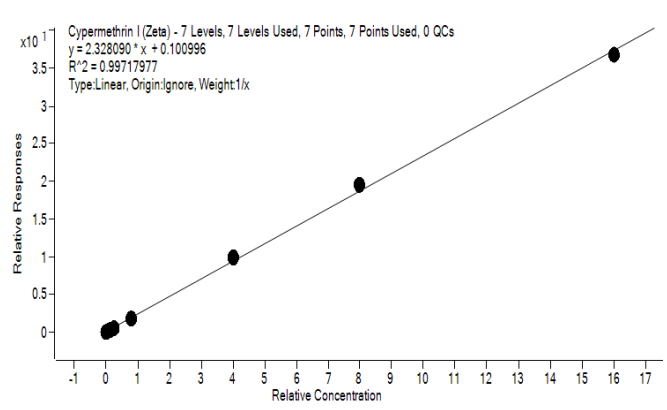
Pyriproxyfen



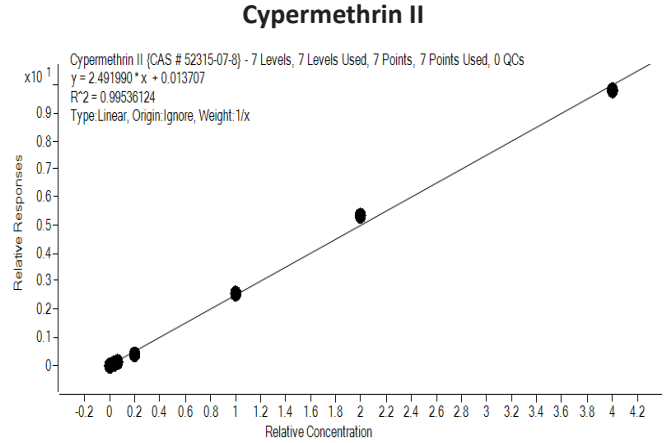
Cyhalothrin -Lambda



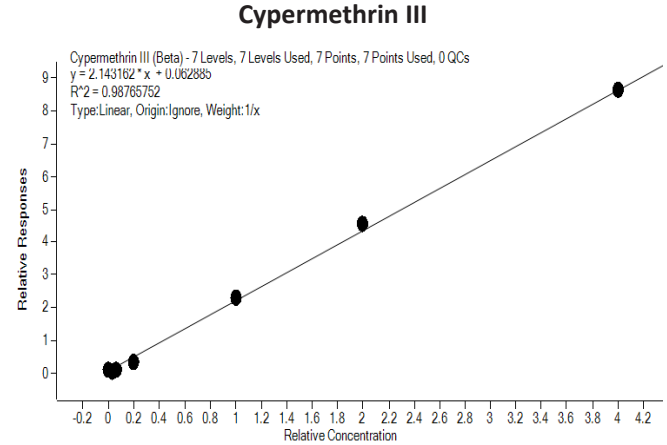
Cypermethrin-I



Cypermethrin II



Cypermethrin III



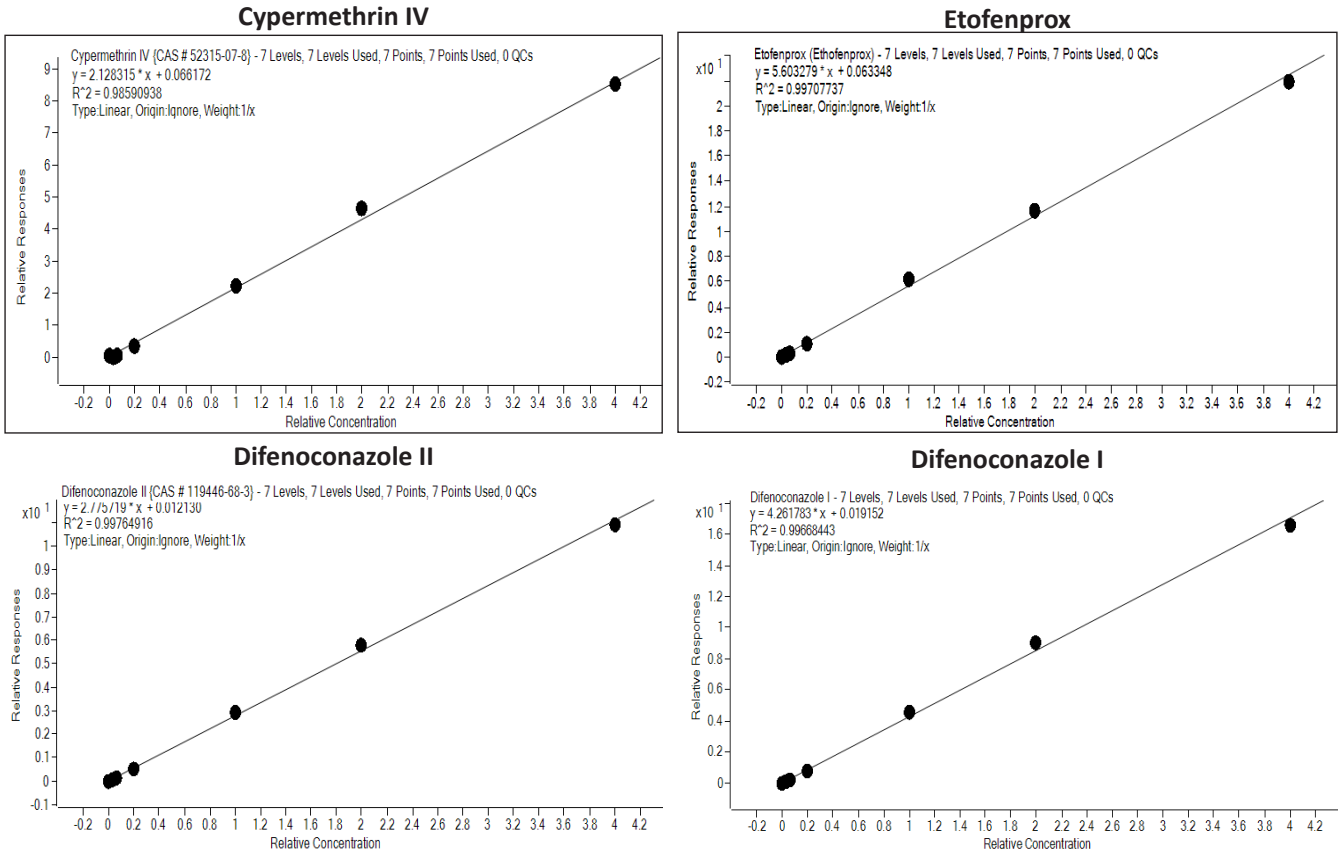


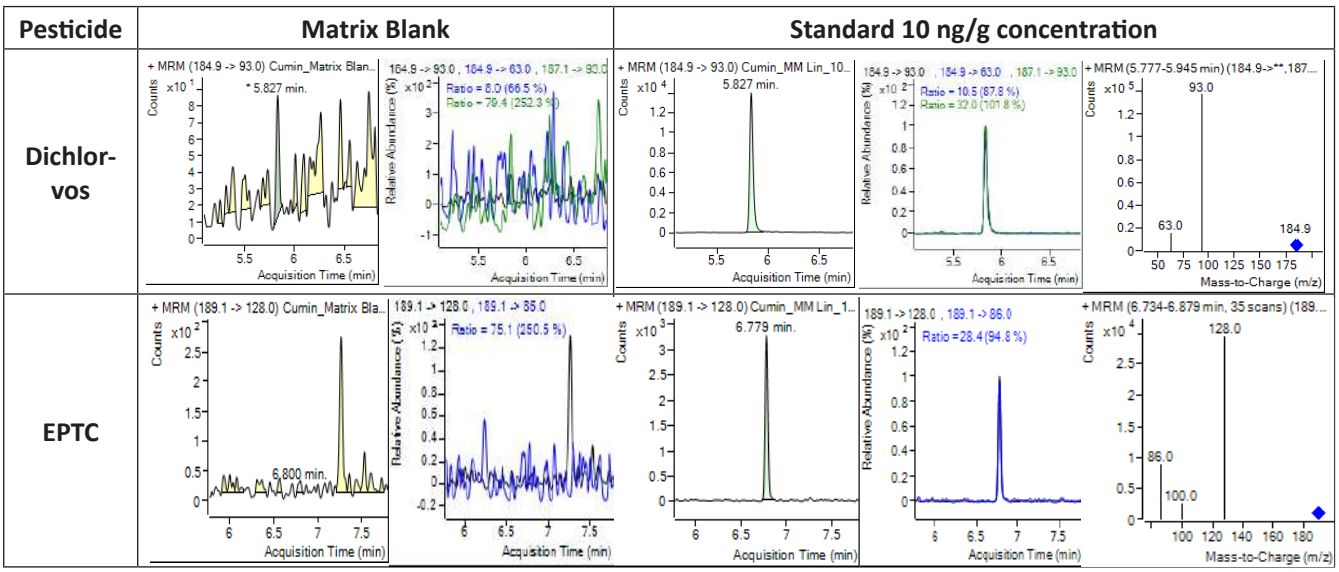
Figure 4. Linearity graphs of 26 pesticides

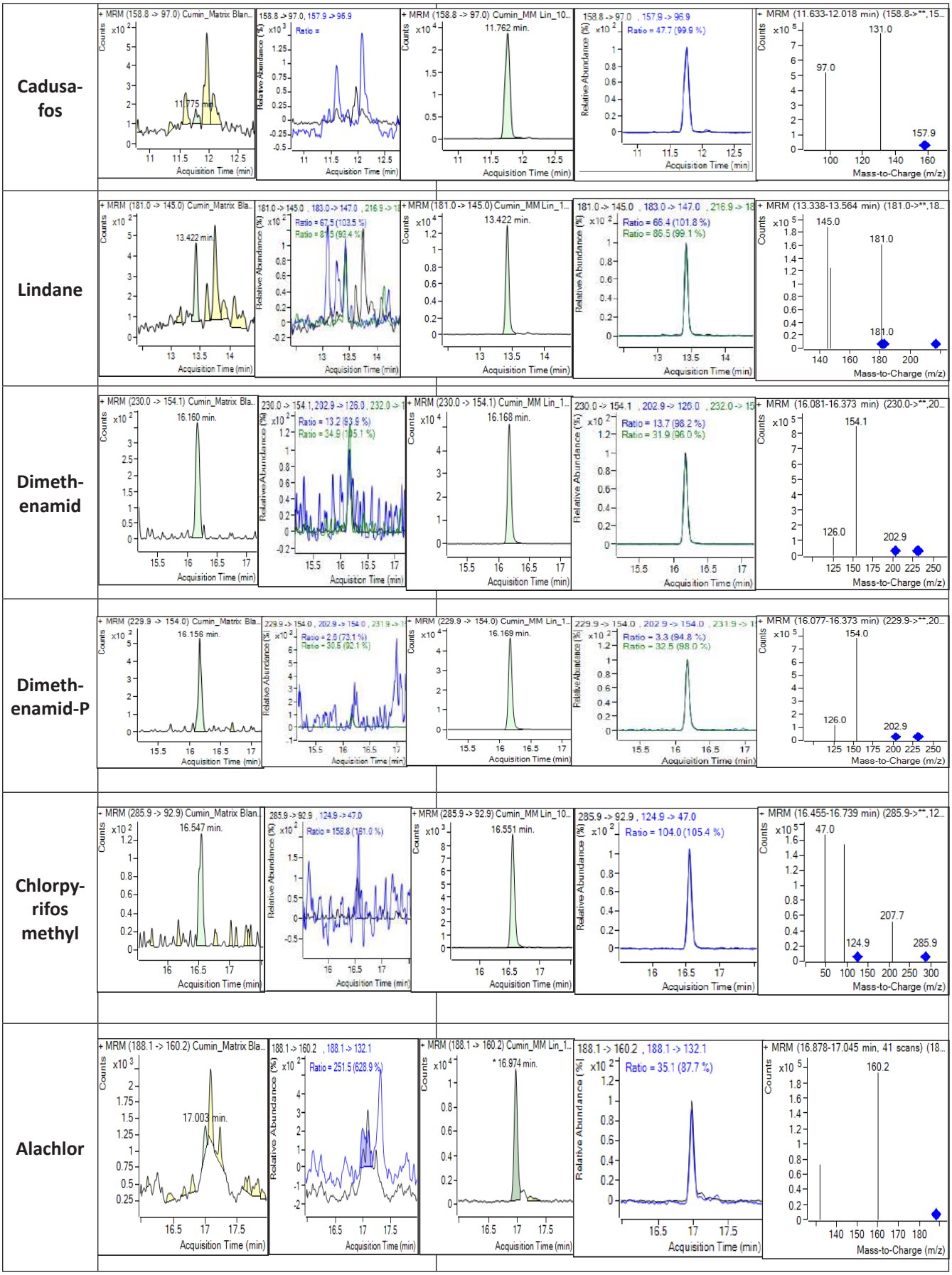
Limit of detection (LOD) and Limit of Quantification (LOQ)

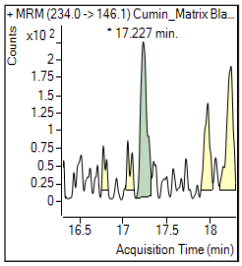
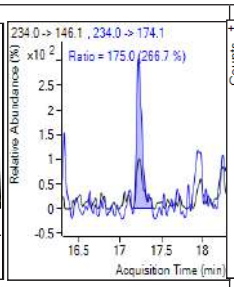
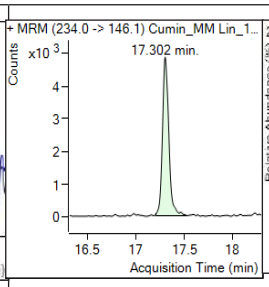
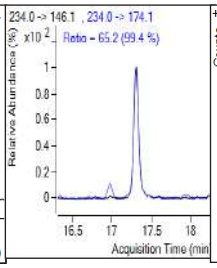
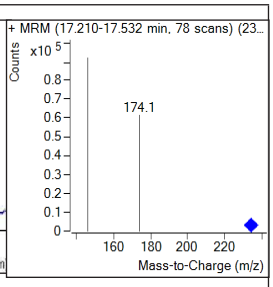
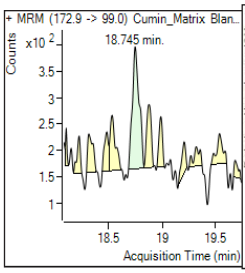
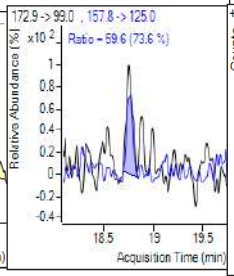
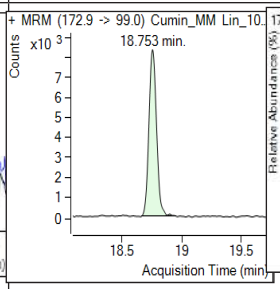
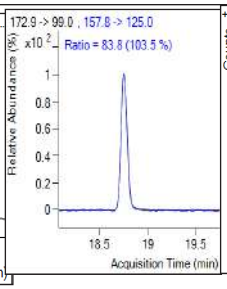
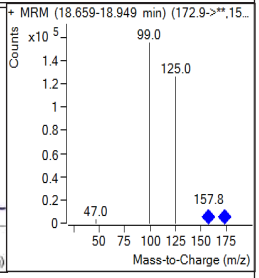
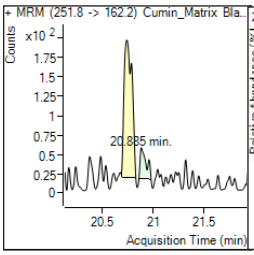
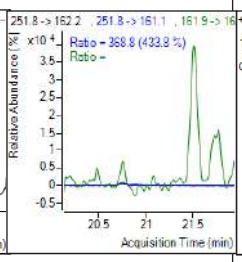
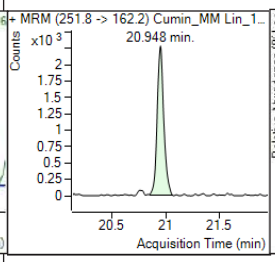
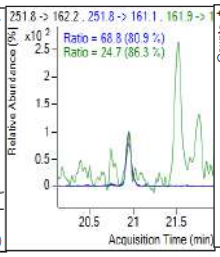
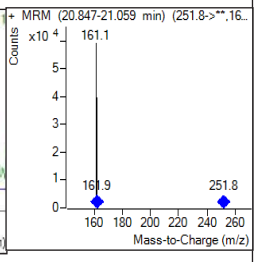
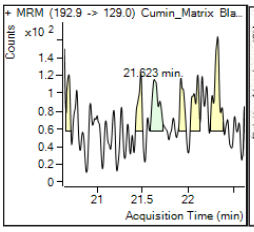
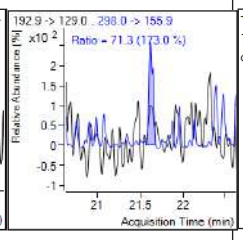
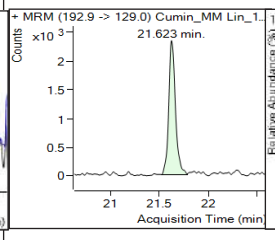
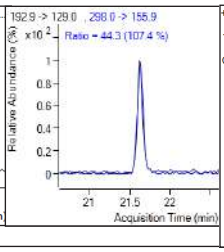
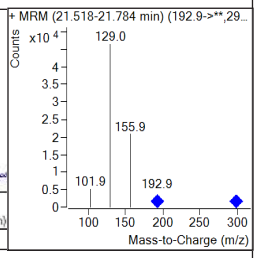
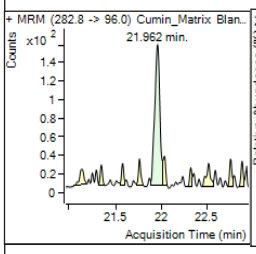
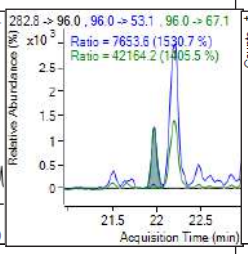
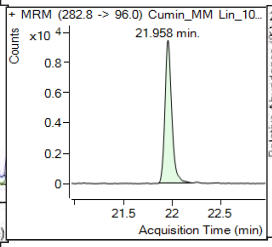
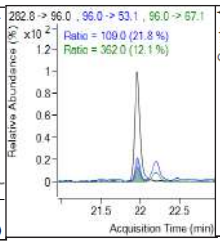
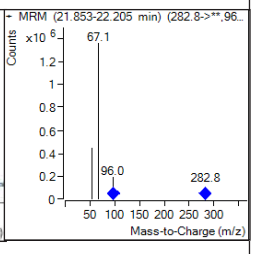
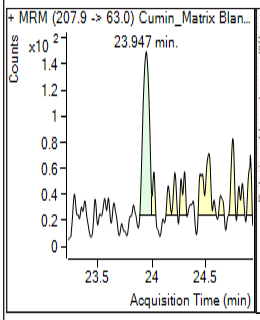
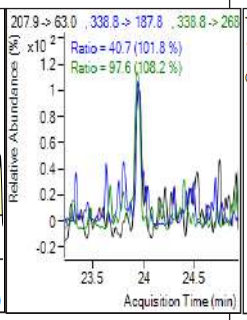
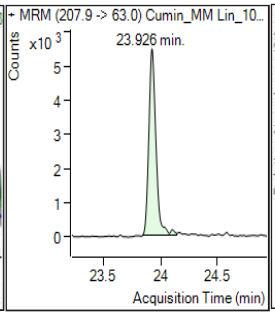
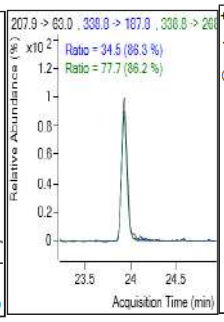
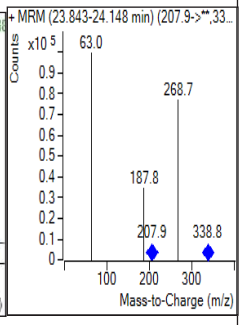
LOD and LOQ of the method for pesticides were evaluated by considering the noise level of the matrix blank cumin sample, with S/N ratio 3:1 and 10:1 respectively. The detection limit for all compounds were calculated as 5 ng/g and lowest quantitation limit was calculated as 10 ng/g respectively. The results of LOQ and LOD complied with the norms of SANTE guidelines for method validation.[22]

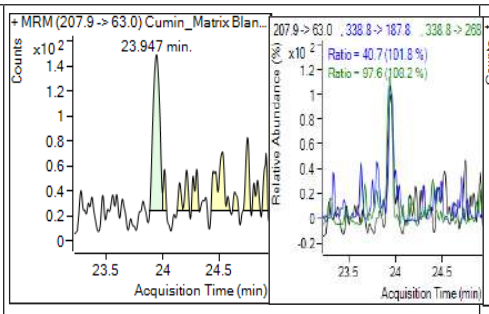
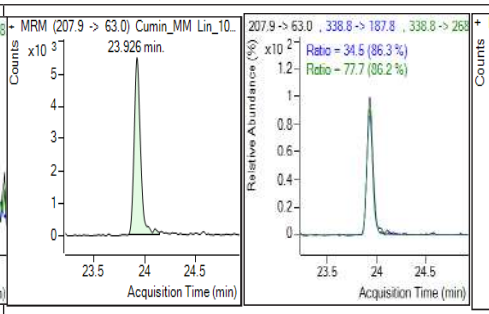
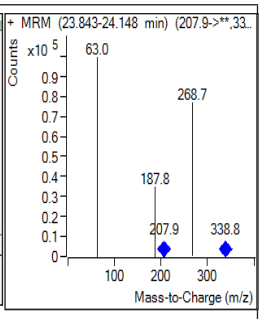
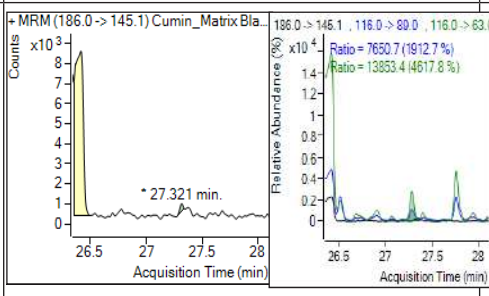
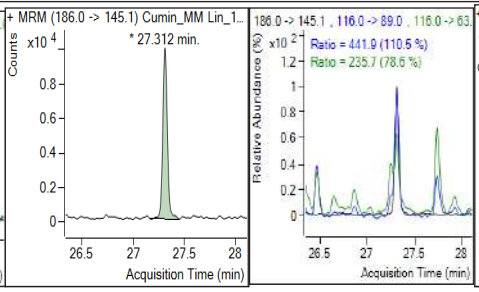
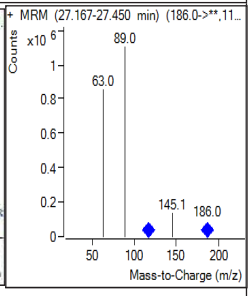
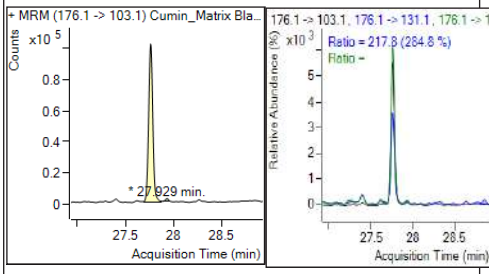
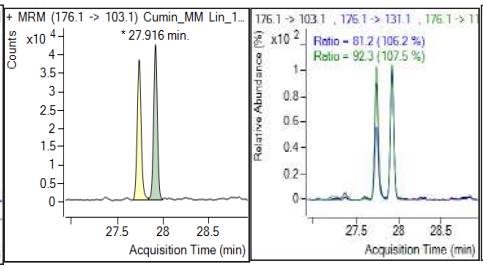
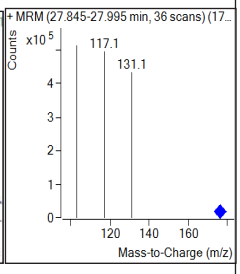
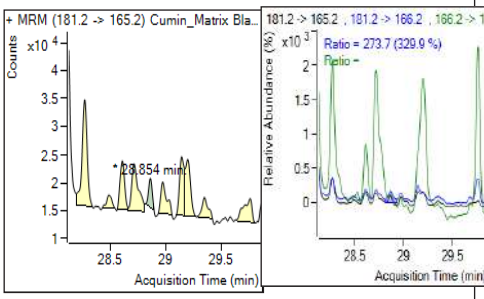
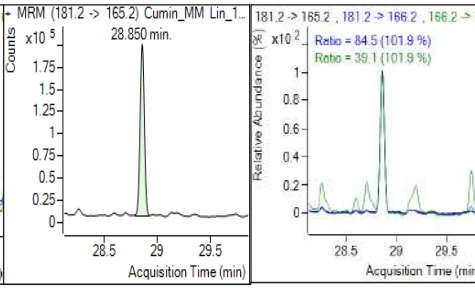
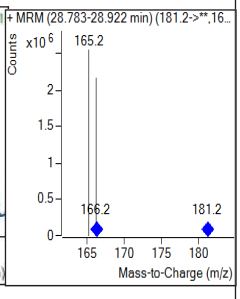
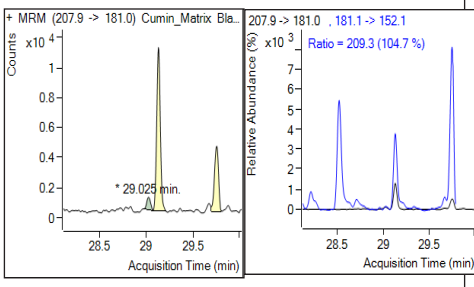
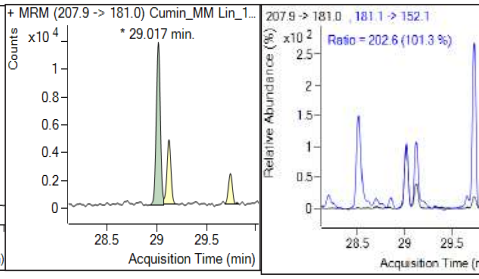
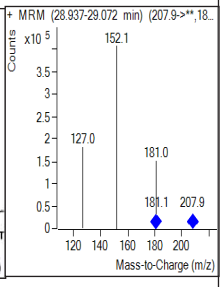
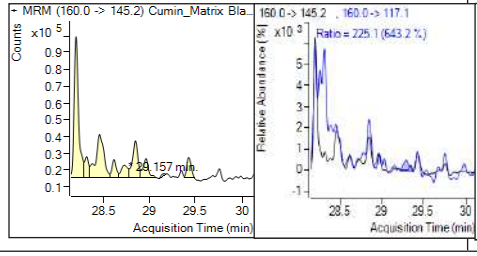
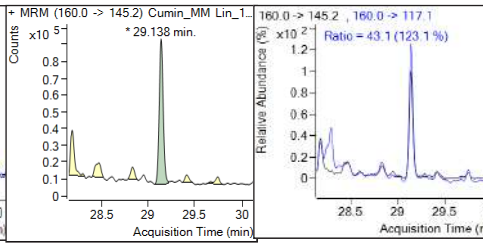
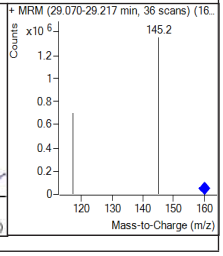
Specificity

The results of the GC-MS/MS chromatograms are presented in Figure-2. For specificity solvent blank, matrix blank, 10 ng/g standard mixture were injected and was checked for interferences in the matrix blank. It was observed that matrix and solvent blank (diluent) was free from interferences. No peaks due to analytes were observed in all the blanks. Results complied with the norms of SANTE guidelines for method validation.[22]




Cadusa-fos
+ MRM (158.8 -> 97.0) Cumin_Matrix Blan...
11.775 min
158.8 -> 97.0, 157.9 -> 96.9
Ratio =
+ MRM (158.8 -> 97.0) Cumin_MM Lin_10...
11.762 min.
158.8 -> 97.0 , 157.9 -> 96.9
Ratio = 47.7 (99.9 %)
+ MRM (11.633-12.018 min) (158.8->**,15...
131.0
97.0
157.9
Lindane
+ MRM (181.0 -> 145.0) Cumin_Matrix Bla...
13.422 min
181.0 -> 145.0 , 183.0 -> 147.0 , 216.9 -> 18
Ratio = 67.5 (103.5 %)
Ratio = 81.5 (93.4 %)
+ MRM (181.0 -> 145.0) Cumin_MM Lin_1...
13.422 min.
181.0 -> 145.0 , 183.0 -> 147.0 , 216.9 -> 18
Ratio = 66.4 (101.8 %)
Ratio = 86.5 (99.1 %)
+ MRM (13.338-13.564 min) (181.0->**,18...
145.0
181.0
181.0
Dimeth-enamid
+ MRM (230.0 -> 154.1) Cumin_Matrix Bla...
16.160 min.
230.0 -> 154.1, 202.9 -> 126.0 , 232.0 -> 1
Ratio = 13.2 (93.8 %)
Ratio = 34.9 (105.1 %)
+ MRM (230.0 -> 154.1) Cumin_MM Lin_1...
16.168 min.
230.0 -> 154.1 , 202.9 -> 126.0 , 232.0 -> 15
Ratio = 13.7 (98.2 %)
Ratio = 31.9 (96.0 %)
+ MRM (16.081-16.373 min) (230.0->**,20...
154.1
126.0
202.9
Dimeth-enamid-P
+ MRM (229.9 -> 154.0) Cumin_Matrix Bla...
16.156 min.
229.9 -> 154.0 , 202.9 -> 154.0 , 231.9 -> 1
Ratio = 2.6 (73.1 %)
Ratio = 30.5 (92.1 %)
+ MRM (229.9 -> 154.0) Cumin_MM Lin_1...
16.169 min.
229.9 -> 154.0 , 202.9 -> 154.0 , 231.9 -> 15
Ratio = 3.3 (94.8 %)
Ratio = 32.5 (98.0 %)
+ MRM (16.077-16.373 min) (229.9->**,20...
154.0
126.0
202.9
Chlorpy-rifos methyl
+ MRM (285.9 -> 92.9) Cumin_Matrix Blan...
16.547 min.
285.9 -> 92.9 , 124.9 -> 47.0
Ratio = 158.8 (151.0 %)
+ MRM (285.9 -> 92.9) Cumin_MM Lin_10...
16.551 min.
285.9 -> 92.9 , 124.9 -> 47.0
Ratio = 104.0 (105.4 %)
+ MRM (16.455-16.739 min) (285.9->**,12...
47.0
207.7
124.9
285.9
Alachlor
+ MRM (188.1 -> 160.2) Cumin_Matrix Bla...
17.003 min.
188.1 -> 160.2 , 188.1 -> 132.1
Ratio = 251.5 (628.9 %)
+ MRM (188.1 -> 160.2) Cumin_MM Lin_1...
16.974 min.
188.1 -> 160.2 , 188.1 -> 132.1
Ratio = 35.1 (87.7 %)
+ MRM (16.878-17.045 min, 41 scans) (18...
160.2
Counts
Relative Abundance (%)
Acquisition Time (min)
Mass-to-Charge (m/z)

Metal-axyl					
Malathi-on					
Pendi-methlin					
Quinal-phos					
Procymi-done					
Profeno-phos					

Profenophos				
Trifloxacin				
Piperonyl butoxide				
Bifenthrin				
Fenpropathrin				
Fenazaquin				

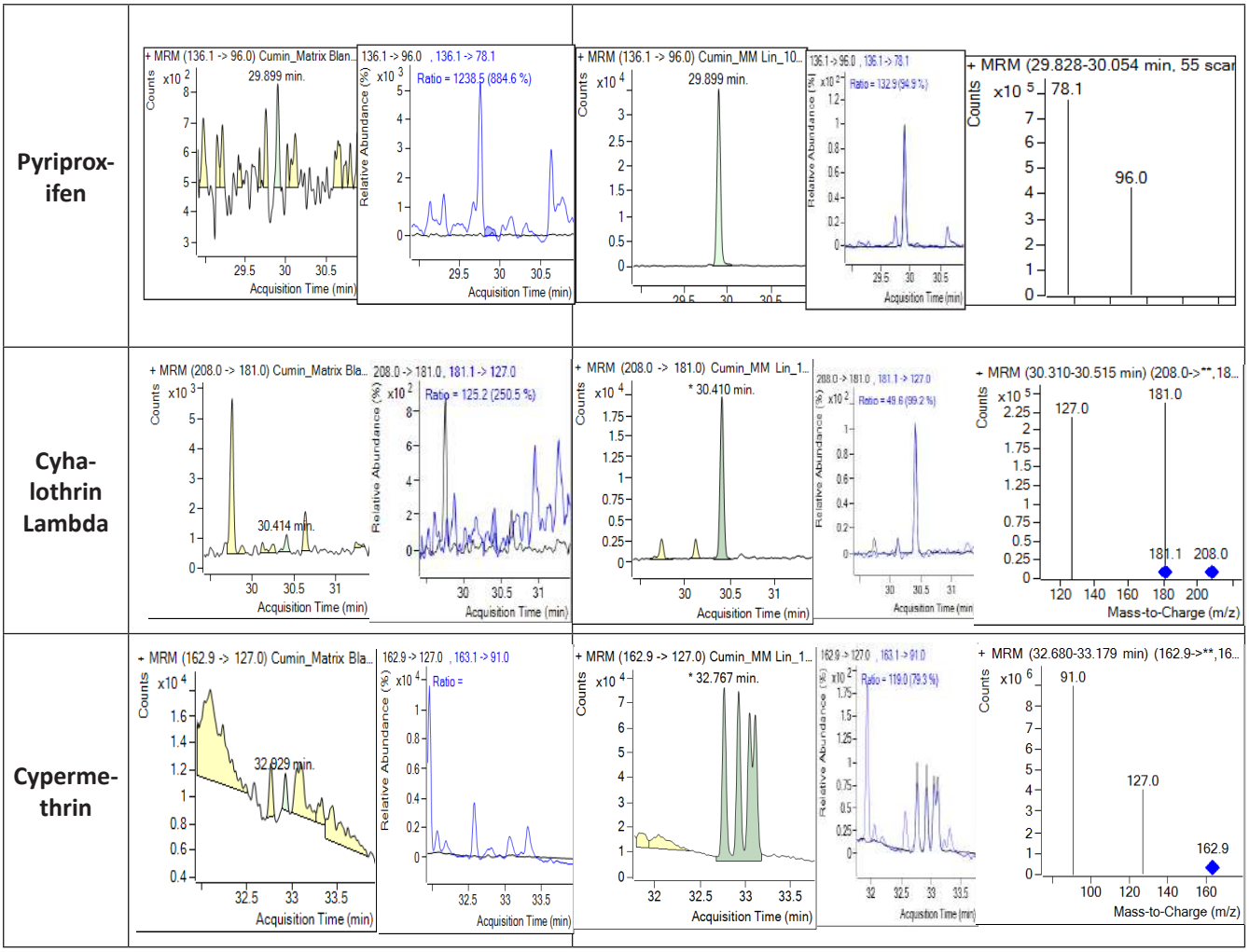


Figure 5.GCMSMS results for matrix blank, standard solutions (10 ng/g) of individual pesticide in standard mi

System Suitability

Standard solutions of pesticide mix of concentration 1.5 ng/g, 3 ng/g, 10 ng/g, 50 ng/g, 100 ng/g and 200 ng/g were prepared in blank cumin matrix. From 1000 ng/g solution of Internal standard (triphenyl phosphate) 0.05 ml was added in each flask to obtain a concentration of 50 ng/g. Final volume was made up to 1 ml. The solutions were injected into GC-MS/MS. Four injections from each solution of concentration 1.5 ng/g and 3 ng/g were injected for area and retention time (RT) check and % RSD was found to be less than 10%. Results complied with the norms of SANTE guidelines for method validation[22] is mentioned in Table 4..

Table 4.System suitability check (Area and RT) for pesticide mix solution of concentration 1.5 ng/g and 3.0 ng/g of matrix matched standards

S. No.	Compound	Retention time	% RSD of concentration 1.5 ng/g		% RSD of concentration 3.0 ng/g	
			Retention time	Area	Retention time	Area
1	Dichlorvos	5.827	0.00	5.00	0.00	1.04
2	EPTC	6.779	0.00	6.81	0.00	8.69
3	Cadusafos	11.767	0.00	7.11	0.00	1.73
4	BHC-gamma	13.418	0.02	2.62	0.02	1.08
5	Dimethenamid	16.168	0.01	9.24	0.01	6.34
	Dimethenamid-P	16.169	0.01	9.75	0.01	3.17
6	Chlorpyrifos-methyl	16.547	0.00	8.62	0.00	0.84

7	Alachlor	16.974	0.01	5.99	0.01	4.50
8	Metalaxyl	17.310	0.01	6.29	0.01	1.11
9	Malathion	18.745	0.03	7.88	0.03	4.51
10	Chlorpyrifos	19.172	0.02	8.87	0.02	4.08
11	Pendimethalin	20.956	0.03	8.83	0.03	3.53
12	Quinalphos	21.631	0.04	8.80	0.04	2.26
13	Procymidone	21.954	0.01	7.51	0.01	5.14
14	Profenofos	23.939	0.01	8.61	0.01	2.32
15	Ethion	25.994	0.03	9.96	0.03	5.57
16	DDE-o,p'	22.453	0.01	8.61	0.01	2.15
	DDE-p,p'	24.008	0.01	9.55	0.01	4.43
	DDD-o,p'	24.352	0.01	9.44	0.01	5.55
	DDD-p,p'	25.726	0.01	7.79	0.01	5.95
	DDT-o,p'	25.730	0.01	8.56	0.01	6.88
	DDT-p,p'	27.027	0.00	3.00	0.00	1.03
17	Trifloxystrobin	27.312	0.01	5.22	0.01	5.65
18	Piperonyl butoxide	27.916	0.01	8.03	0.01	5.99
19	Bifenthrin	28.850	0.00	9.33	0.00	7.47
20	Fenpropathrin	29.017	0.01	8.45	0.01	4.07
21	Fenazaquin	29.138	0.01	9.35	0.01	1.50
22	Pyriproxyfen	29.899	0.01	7.52	0.01	7.87
23	Cyhalothrin (lambda)	30.410	0.01	9.98	0.01	11.01
24	Cypermethrin mix	32.763	0.01	8.28	0.01	4.46
	Cypermethrin II	32.925	0.00	6.45	0.00	6.22
25	Etofenprox	33.224	0.01	5.73	0.01	4.64
26	Difenoconazole mix	35.386	0.01	7.92	0.01	7.42
	Difenoconazole II	35.231	0.00	7.37	0.00	3.15

Accuracy (Recovery studies)

The recoveries of each pesticide in spiked samples were calculated. The recovery studies of pesticide mix were carried out for spiked level 5.0 and 10.0 ng/g concentrations of cumin samples respectively, then prepared the samples. The solutions were injected in six replicates and GC-MS/MS analysis method was used as mentioned above. The recoveries of pesticides in cumin samples were observed to be in the range of 70.74 to 117.56% (Table 5 and 6). In terms of repeatability, the majority of pesticides gave RSD < 20%. The recoveries and repeatability were in accordance with the criteria set by SANTE Guidelines.[22]

Table 5.Recovery data for the proposed method for pesticide mix in samples of cumin at concentration of 5 ng/g

Recovery Calculation for Cumin, Spiking level: 10 ng/g										
Concentration, ng/g										
S.No	**Compound**	**Vial 1**	**Vial 2**	**Vial 3**	**Vial 4**	**Vial 5**	**Vial 6**	**Avg**	**Result (mg/kg)**	**% Recovery**
1	Dichlorvos	7.03	7.23	7.01	7.00	7.01	7.17	7.07	0.01	70.74
2	EPTC	7.04	7.14	7.05	7.01	7.02	7.22	7.08	0.01	70.80
3	Cadusafos	9.88	10.00	9.50	10.23	10.17	9.91	9.95	0.01	99.49
4	BHC-gamma	9.54	8.91	9.41	9.27	9.67	9.23	9.34	0.01	93.37

5	Dimethenamid	4.30	5.31	4.62	4.48	4.99	5.29	4.83	0.0048	96.65
	Dimethenamid-P	4.35	5.27	4.52	4.47	5.09	5.27	4.83	0.0048	96.60
6	Chlorpyrifos-methyl	3.81	4.87	4.76	4.42	4.65	4.62	4.52	0.0045	90.41
7	Alachlor	5.34	5.77	5.71	5.56	5.61	5.95	5.66	0.0057	113.16
8	Metalaxyl	4.77	5.47	4.79	4.61	5.33	5.76	5.12	0.0051	102.46
9	Malathion	3.74	4.63	4.37	4.21	4.18	4.55	4.28	0.0043	85.59
10	Chlorpyrifos	5.49	5.93	5.83	5.91	5.93	5.96	5.84	0.0058	116.82
11	Pendimethalin	4.63	4.91	5.10	4.86	5.53	5.29	5.05	0.0051	101.06
12	Quinalphos	3.60	4.51	4.48	4.30	4.31	4.46	4.28	0.0043	85.58
13	Procymidone	3.87	4.72	4.23	4.79	4.77	4.41	4.47	0.0045	89.32
14	Profenofos	4.14	4.62	4.51	4.12	4.40	4.19	4.33	0.0043	86.61
15	Ethion	3.64	4.44	3.99	4.07	4.39	4.38	4.15	0.0042	83.06
16	DDE-o,p’	3.60	4.16	4.20	3.60	4.56	4.56	4.11	0.0041	82.27
	DDE-p,p’	3.65	3.70	3.66	3.56	3.58	3.92	3.68	0.0037	73.59
	DDD-o,p’	3.53	4.38	3.55	3.62	4.23	4.45	3.96	0.0040	79.26
	DDD-p,p’	3.76	4.60	4.04	3.76	4.57	4.55	4.21	0.0042	84.25
	DDT-o,p’	3.73	4.25	4.15	3.69	4.62	4.56	4.17	0.0042	83.37
	DDT-p,p’	4.02	4.35	3.90	3.98	4.97	4.19	4.23	0.0042	84.70
17	Trifloxystrobin	5.29	5.91	5.94	5.95	5.94	5.97	5.83	0.0058	116.68
18	Piperonyl butoxide	4.41	4.85	4.46	4.56	4.85	5.33	4.74	0.0047	94.86
19	Bifenthrin	4.12	4.49	4.88	4.28	4.66	5.06	4.58	0.0046	91.63
20	Fenpropathrin	4.78	5.10	5.69	5.18	5.62	4.79	5.19	0.0052	103.88
21	Fenazaquin	4.47	5.01	4.77	4.58	5.29	5.12	4.87	0.0049	97.47
22	Pyriproxyfen	4.18	4.94	4.25	4.46	4.85	5.04	4.62	0.0046	92.40
23	Cyhalothrin (lambda)	3.53	4.40	3.71	3.60	3.62	4.42	3.88	0.0039	77.59
24	Cypermethrin mix	17.66	18.77	17.74	17.94	17.34	18.91	18.06	0.0181	90.31
	Cypermethrin II	4.35	4.87	4.03	4.09	3.88	4.78	4.33	0.0043	86.68
25	Etofenprox	3.55	4.24	3.61	3.62	4.31	4.35	3.94	0.0039	78.88
26	Difenoconazole mix	4.12	5.16	4.42	4.19	4.92	6.87	4.94	0.0049	98.88
	Difenoconazole II	4.22	5.10	4.47	4.22	4.63	5.04	4.61	0.0046	92.26

Table 6.Recovery data for the method for pesticide mix in samples of cumin at concentration of 10 ng/g

Recovery Calculation for Cumin, Spiking level: 10 ng/g										
Concentration, ng/g										
S.No	**Compound**	**Vial 1**	**Vial 2**	**Vial 3**	**Vial 4**	**Vial 5**	**Vial 6**	**Avg**	**Result (mg/kg)**	**% Recovery**
1	Dichlorvos	7.03	7.23	7.01	7.00	7.01	7.17	7.07	0.01	70.74
2	EPTC	7.04	7.14	7.05	7.01	7.02	7.22	7.08	0.01	70.80

3	Cadusafos	9.88	10.00	9.50	10.23	10.17	9.91	9.95	0.01	99.49
4	BHC-gamma	9.54	8.91	9.41	9.27	9.67	9.23	9.34	0.01	93.37
5	Dimethenamid	9.98	10.20	9.26	9.98	10.01	9.59	9.84	0.01	98.37
	Dimethenamid-P	9.65	9.81	9.49	9.67	9.84	9.58	9.67	0.01	96.74
6	Chlorpyrifos-methyl	10.37	9.61	9.38	9.32	9.34	9.70	9.62	0.01	96.18
7	Alachlor	11.13	11.90	11.42	10.59	11.98	9.88	11.15	0.01	111.51
8	Metalaxyl	11.11	10.64	10.25	11.52	11.27	10.27	10.84	0.01	108.42
9	Malathion	10.06	10.79	10.04	9.52	10.42	10.14	10.16	0.01	101.60
10	Chlorpyrifos	11.44	11.85	11.75	11.74	11.87	11.88	11.76	0.01	117.56
11	Pendimethalin	9.31	9.88	9.84	11.01	9.41	8.82	9.71	0.01	97.13
12	Quinalphos	9.79	9.39	10.03	8.10	10.71	9.90	9.65	0.01	96.53
13	Procymidone	9.65	9.76	9.28	9.67	9.75	9.92	9.67	0.01	96.72
14	Profenofos	10.18	10.22	9.12	9.25	9.80	10.32	9.82	0.01	98.16
15	Ethion	9.06	8.95	8.24	8.98	8.26	8.35	8.64	0.01	86.38
16	DDE-o,p’	9.25	8.91	8.26	8.37	8.45	8.35	8.60	0.01	85.99
	DDE-p,p’	7.32	7.75	6.53	6.82	6.89	6.69	7.00	0.01	69.98
	DDD-o,p’	8.98	9.39	8.14	8.86	8.71	8.26	8.72	0.01	87.25
	DDD-p,p’	9.06	9.25	8.49	8.86	8.81	8.76	8.87	0.01	88.71
	DDT-o,p’	9.18	9.13	8.33	8.90	9.07	8.49	8.85	0.01	88.51
	DDT-p,p’	8.54	8.50	7.83	8.19	7.80	8.19	8.18	0.01	81.75
17	Trifloxystrobin	10.75	11.45	10.56	11.71	11.32	11.03	11.14	0.01	111.38
18	Piperonyl butoxide	10.08	10.46	9.99	9.29	9.77	9.65	9.87	0.01	98.71
19	Bifenthrin	8.96	9.74	7.87	8.32	9.00	8.63	8.75	0.01	87.54
20	Fenpropathrin	10.49	10.43	10.15	9.18	10.91	9.41	10.09	0.01	100.94
21	Fenazaquin	8.75	9.08	9.08	7.88	8.67	8.22	8.61	0.01	86.11
22	Pyriproxyfen	9.65	9.32	8.81	9.33	9.76	9.13	9.33	0.01	93.33
23	Cyhalothrin (lambda)	8.64	9.06	7.36	8.35	8.31	8.99	8.45	0.01	84.51
24	Cypermethrin mix	34.93	35.87	34.39	35.72	34.98	34.81	35.12	0.04	87.79
	Cypermethrin II	8.91	9.35	9.04	10.15	9.72	9.31	9.41	0.01	94.11
25	Etofenprox	9.04	9.22	8.34	8.84	8.67	8.54	8.78	0.01	87.76
26	Difenoconazole mix	10.34	11.81	9.75	9.86	10.42	9.26	10.24	0.01	102.42
	Difenoconazole II	10.16	11.83	9.61	9.84	10.36	9.24	10.17	0.01	101.73

Precision Studies

The repeatability measurement in the study in terms of precision was conducted by measuring the concentrations in six replicates as presented in Table 7 and 8. Pesticide blank samples were spiked at two different concentrations 5.0 and 10 ng/g. The relatively lower standard deviation (RSD) values were obtained within the acceptable limits, indicating the precision of the developed method, thus, it can be adopted for analysis. Results complied with the norms of SANTE guidelines for method validation.[22]

While performing system suitability check for area and retention time (RT), % RSD of area of concentration 1.5 ng/g was found to be maximum (9.98%) in pesticide cyhalothrin lambda and minimum in chlorpyrifos methyl (0.84%). Recovery % for the pesticides were according to SANTE guidelines and were found to be in the range 77 to 117%.

Precision data so obtained showed % RSD to be less than 10 %.[22,23,24,25] Collision energy was optimized for each pesticide. Matrix effect (co-interferences) was reduced by making linearity in matrix match standards. Spice cumin is reported to have 6.1 % moisture therefore addition of water and keeping for 30 minutes prior to sample extraction analysis improved recovery % .[26-27]

Table 7.Precision data for the method of pesticide residues in cumin samples at concentration level of 5 ng/g

Spiking level: 5 ng/g										
S.No.	**Compound**	**Vial 1**	**Vial 2**	**Vial 3**	**Vial 4**	**Vial 5**	**Vial 6**	**Avg**	**Std Dev**	**% RSD**
1	Dichlorvos	3692	3889	4408	4555	4344	4090	4163	331	7.96
2	EPTC	773	794	975	890	984	877	882.06	87	9.97
3	Cadusafos	15794	19856	19469	19531	20253	21158	19343.81	1845	9.54
4	BHC-gamma	7011	8406	8532	7211	8496	8714	8061.70	745	9.25
5	Dimethenamid	33286	39868	35250	33849	39477	41967	37282.72	3614	9.70
	Dimethenamid-P	30547	36960	32279	31585	36167	39097	34439.25	3435	9.97
6	Chlorpyrifos-methyl	5512	6972	6898	6369	6899	7024	6612.45	588	8.90
7	Alachlor	9354	10105	10102	9767	10164	10980	10078.86	537	5.33
8	Metalaxyl	4487	5034	4596	4418	5084	5541	4723.93	313	6.63
9	Malathion	6728	8181	7853	7511	7714	8508	7749.33	612	7.90
10	Chlorpyrifos	16663	17646	17637	17586	18203	18721	17742.62	689.	3.89
11	Pendimethalin	2006	2136	2235	2115	2459	2419	2228.33	179	8.04
12	Quinalphos	1784	2186	2193	2096	2168	2289	2119.21	175	8.29
13	Procymidone	6200	7509	6865	7610	7823	7464	7245.22	603	8.33
14	Profenofos	4059	4518	4467	4078	4459	4386	4327.76	205	4.74
15	Ethion	15157	17667	16501	16561	18040	18487	17068.98	1228	7.20
16	DDE-o,p’	27442	30954	31516	27673	34350	35236	31195.01	3253	10.43
	DDE-p,p’	27549	28078	28155	27318	28344	30945	28398.36	1306	4.60
	DDD-o,p’	36081	43404	36966	27673	43535	46559	39036.25	6900	17.68
	DDD-p,p’	64190	76973	69464	64676	78888	80698	72481.56	7314	10.09
	DDT-o,p’	129544	146091	144379	129281	161680	163976	145824.99	14978	10.27
	DDT-p,p’	50921	54907	50702	51038	63292	56293	54525.38	4896	8.98
17	Trifloxystrobin	6290	7023	7119	7057	7274	7487	7041.84	405	5.76
18	Piperonyl butoxide	22967	25069	23655	23811	25843	28671	25002.82	2075	8.30
19	Bifenthrin	81805	89060	96703	85410	94806	104551	92055.85	8288	9.00
20	Fenpropathrin	7474	7894	8603	7967	8717	7985	8106.61	468	5.78
21	Fenazaquin	39794	44513	42989	40967	48198	47943	44067.39	3501	7.95
22	Pyriproxyfen	14998	17761	15544	16092	18022	19163	16930.01	1626	9.61
23	Cyhalothrin (lambda)	8185	9858	8680	8394	8701	10461	9046.59	903	9.99
24	Cypermethrin mix	169550	178975	173489	173031	174381	190225	176608.63	7325	4.15
	Cypermethrin II	38439	42662	36837	36910	36480	44438	39294.15	3411	8.68
25	Etofenprox	88970	101375	91809	90963	105713	109168	97999.82	8543	8.72

26	Difenoconazole mix	64832	74524	69883	65311	73705	97462	74286.15	12056	16.23
	Difenoconazole II	40235	47947	43137	40575	45403	50102	44566.51	3987	8.95

Table 8.Precision data for the method of pesticide residues in cumin samples at concentration level of 10 ng/g

Spiking level: 10 ng/g										
S. No.	**Compound**	**Vial 1**	**Vial 2**	**Vial 3**	**Vial 4**	**Vial 5**	**Vial 6**	**Avg**	**Std Dev**	**% RSD**
1	Dichlorvos	7414	7455	7574	7386	7223	7660	7452	153	2.05
2	EPTC	1518	1504	1556	1511	1478	1575	1524	36	2.34
3	Cadusafos	41587	41159	40970	43002	41788	42252	41793	748	1.79
4	BHC-gamma	14417	13248	14585	14032	14247	14172	14117	467	3.31
5	Dimethe-namid	33286	39868	35250	33849	39477	41967	37282.72	3614	9.70
	Dimethe-namid	74605	74550	71046	74529	73056	72665	73408	1432	1.95
	Dimethe-namid-P	67180	66733	67711	67301	66914	67560	67233	373	0.55
6	Chlorpyrifos-methyl	14482	13168	13476	13063	12797	13760	13458	603	4.48
7	Alachlor	18635	19395	19550	17781	19495	16892	18625	1088	5.84
8	Metalaxyl	9189	8651	8776	9480	9088	8694	8980	328	3.65
9	Malathion	16821	17569	17203	15969	16991	17166	16953	543	3.20
10	Chlorpyrifos	28246	28371	29529	28786	28364	29436	28789	569	1.98
11	Pendimethalin	3969	4107	4287	4660	3917	3815	4126	309	7.48
12	Quinalphos	4463	4199	4679	3746	4742	4571	4400	373	8.48
13	Procymidone	14974	14806	14779	15002	14781	15581	14987	307	2.05
14	Profenofos	9499	9326	8770	8676	8951	9744	9161	428	4.67
15	Ethion	31643	30635	29941	31387	28590	29914	30352	1121	3.69
	16	27549	28078	28155	27318	28344	30945	28398.36	1306	4.60
	DDE-o,p'	61669	58314	57138	56439	55623	57066	57708	2133	3.70
	DDE-p,p'	47852	49056	44715	45156	44495	45063	46056	1911	4.15
	DDD-o,p'	82746	84277	77559	81655	78574	77673	80414	2862	3.56
	DDD-p,p'	143936	143461	138820	140934	137053	141294	140916	2648	1.88
	DDT-o,p'	295045	287131	276357	286363	285029	278006	284655	6785	2.38
	DDT-p,p'	100061	97388	94787	96340	90134	97539	96041	3370	3.51
17	Trifloxystrobin	12390	12860	12483	13419	12700	12852	12784	365	2.86
18	Piperonyl butoxide	48171	48704	48957	44721	45738	46892	47197	1713	3.63
19	Bifenthrin	168717	178262	153337	157424	165524	165004	164711	8751	5.31
20	Fenpropathrin	13910	13540	13877	12481	14043	12898	13458	632	4.69
21	Fenazaquin	75552	76523	80200	68463	73224	72181	74357	4026	5.41
22	Pyriproxyfen	34500	32629	32356	33381	34094	33128	33348	829	2.49

23	Cyhalothrin (lambda)	17861	18229	15872	17309	16848	18750	17478	1033	5.91
24	Cypermethrin mix	295422	295356	298819	300770	288960	298351	296280	4149	1.40
25	Cypermethrin II	73743	75391	76541	83000	77976	77760	77402	3161	4.08
26	Etofenprox	184220	183225	176697	180756	173890	177965	179459	3985	2.22
27	Difenoconazole mix	142444	x157771	138120	136146	140078	130207	140794	9298	6.60
	Difenoconazole II	91057	102726	88570	88362	90592	84417	90954	6227	6.85

Discussion

When the analysis is carried out using matrix match internal standard, it is generally reported that matrix adversely affected quantification of pesticides at residue levels. Co-extracted interferences were ascribed to the challenges in arriving at the true value of the data of analysis.[28] There are ways by which matrix affects can be reduced or prevented such as a) matrix matched calibration method b) addition of internal standard c) use of analyte protectants, d) suitable modifications in the method of sample preparation etc. In the present study, spiked matrix matched calibration curves were constructed and internal standard was added in order to overcome the problems caused by matrix affect. Calibration matrix match standards were made by the addition of standard solution to blank cumin samples and the samples were subjected to the same sample preparation procedure intended to be used for unknown samples. By doing this, the composition of both standard sample matrices and unknown samples would be same and effect of matrix will be seen in both standards and unknown samples. The calibration curve was constructed using spiked matrix matched standards and concentration of analytes in samples could be easily calculated without any interference due to the matrix affects. The present method is unique as it has taken care of all the challenges due to the presence of complex matrices. The recoveries and repeatability were in accordance with SANTE guidelines. The results of all the validation parameters also satisfy the requirements of a standard method that can be easily adopted.

Conclusion

A simple multi-residue method for the simultaneous analysis of 26 pesticides in seed spice cumin using gas chromatography mass spectrometry (GC-MS/MS) was developed and validated. Sample preparation method involved use of modified QuEChERS method which is a dispersive solid phase extraction technique. In this study, after initial extraction by shaking with acidified acetonitrile (1% acetic acid) magnesium sulphate and sodium chloride were added for salting out effect. Buffers like trisodium citrate dihydrate and disodium hydrogen citrate sesquihydrate were used to maintain the pH of the sample solution in the range 5 to 5.5. Primary secondary amine (PSA) and Graphitized carbon black (GCB) sorbents were used to remove polar co extractives like organic acids, sugars, fatty acids etc. A quick, easy and economic method has been successfully developed for some pesticides in cumin samples [29,30]. The validated method is highly precise (% RSD less than 20 %), accurate (recovery 70 to 120 %), and sensitive (LOQ 10 ng/g and LOD 5.0 ng/g) for determination of pesticide mix in cumin samples. Since, pesticide residues analysis is most challenging in spices due to high interferences present while developing a method the following steps should be taken care of: a) Sample processing (mixing, blending and homogenization) of sample b) Extraction of analyte of interest from complex matrix having interferences or co-analytes c) Clean up and derivatization (if required) to minimize interferences d) Estimation which includes detection and quantification of analyte of interest using various analytical techniques. The validation results satisfied the SANTE /12682/2019 guidelines. Limit of quantification was set at 10 ng/g for all the analytes of pesticides.

References

1. Muyesaier Tudi, Huada Daniel Ruan, Li Wang, Jia Lyu, Ross Sadler, Des Connell, Cordia Chu and Dung Tri Phung, Agriculture Development, Pesticide Application and Its Impact on the Environment, International Journal of Environmental Research and Public Health, 2021 Feb, 18(3): 1112, doi: 10.3390/ijerph18031112
2. Ajmer Singh Grewal, Ashish Singla, Pradeep Kamboj, Jagdeep Singh Dua, Pesticide Residues in Food Grains, Vegetables, and Fruits: A Hazard to Human Health, Journal of Medicinal Chemistry and Toxicology, 2017, 20 March, 2(1):1-7
3. Jamaludheen A, P.K Singh, V.K Choudhary, Yogita Gharde, J.S. Mishra, Herbicides vis-à-vis other pesticides: Trend analysis and economic impact, ICAR- Directorate of Weed Research, Jabalpur, Technical Bulletin: 23, https://dwr.icar.gov.in/

4. Vinay Mohan Pathak, Vijay K. Verma, Balwant Singh Rawat, Baljinder Kaur, Neelesh abu, Akansha Sharma, Seeta Dewali, Monika Yadav, Reshma Kumari, Sevaram Singh, Asutosh Mohapatra, Varsha Pandey, Nitika Rana, and Jose Maria Cunill, Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review, Front Microbiol. 2022; 17 August, 13: 962619. doi: 10.3389/fmicb.2022.962619
5. Md. Wasim Aktar, Dwaipayan Sengupta and Ashim Chowdhury, Impact of pesticides use in Agriculture: their benefits and hazards, Interdisciplinary Toxicology, National Library of Medicine, 2009 Mar, vol-2 (1): 1-12, doi: 10.2478/v10102-009-0001-7
6. R Geetha, T Ravisankar, P.K Patil, Satheesha Avunje, S. Vinoth, C.V Sairam and K.K Vijayan, "Trends, causes, and indices of import rejections in international shrimp trade with special reference to India: a 15-year longitudinal analysis", Aquaculture international, 2020 13 April, 28, 1341–1369,
7. Virat Bahri , MRL: A bane for India's agricultural export ambitions, Trade promotion Council of India, 2020, December 17
8. Mohit Kumar, Pramod Kumar Sahoo, Dilip Kumar Kushwaha, Shailendra Nath Saxena, Indra Mani, Gurjeet Singh and Nrusingh Charan Pradhan, Cumin cultivation: Present status and future prospects, The pharma Innovation Journal, 2021, SP-10(8):1121-1131
9. Shinoj Parrapurathu, V C Mathur, Analysis for demand for major spices in India, , Agricultural Economics Research Review, ICAR, Jan 2006,Vol 19, July-December 2006, pp 367-376
10. A. Minhas, Cumin production volume of spices India FY, 2009-2022
11. Muhammad Nadeem, Asad Riaz, Cumin (Cuminum cyminum) as a potential source of antioxidants, Jiangsu Academy of Agricultural Sciences, National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan, PAK. J. FOOD SCI., 22(2), 2012:101-107, ISSN: 2226-5899
12. Kanagal Sahana, Subban Nagarajan, Lingamallu Jagan Mohan Rao, Cumin (Cuminum cyminum L.) Seed Volatile Oil: Chemistry and Role in Health and Disease Prevention, Nuts and Seeds in Health and Disease Prevention2011, Pages 417-427, https://doi.org/10.1016/B978-0-12-375688-6.10050-7
13. Department of Agriculture & Farmers Welfare, Ministry of Agriculture, Annual Report-2021-2022, https://www.bing.com/ck/a?!&&p=e64650fd69bb5f10JmltdHM9MTY5MTE5MzYwMCZpZ3VpZD0yNTkyNjAyNy01MjNhLTY0ZWQtMTkzNC03MTMzNTM4ODY1M2ImaW5zaWQ9NTE5OA&ptn=3&hsh=3&fclid=259260-27-523a-64ed-1934-71335388653b&psq=13)%09Department+of+Agriculture+%26+Farmers+Welfare%2c+Ministry+of+Agriculture%2c+Annual+Report+-2021-2022+&u=a1aHR0cHM6Ly9hZ3JpY29vcC5uaWMuaW4vZW4vQW5udWFs&ntb=1
14. Rachana Bansal, Mohit Kumar and A. S. Shaikh, Growth and export trends of major spices in India: An Analytical study, Guj. J. Ext. Edu. Vol. 33: Issue 2: June 2022, https://doi.org/10.56572/gjoee.2022.33.2.0028
15. Ministry of commerce and industry, Government of India, Annual Report, 2021-2022, https://www.bing.com/ck/a?!&&p=194526090be1c474JmltdHM9MTY5MTE5MzYwMCZpZ3VpZD0yNTkyNjAyNy01MjNhLTY0ZWQtMTkzNC03MTMzNTM4ODY1M2ImaW5zaWQ9NTE5Ng&ptn=3&hsh=3&fclid=25926027-523a-64ed-1934-71335388653b&psq=Ministry+of+Commerce+and+Industry%2c+Government+of+India%2c+the+total+exports+of+cumin+seeds+during+2020-2021+was+%7e+USD+185+million.+The+value+(million+USD)+of+cumin+seeds+exported+to+top+ten+countries+were%3a+i)+China%2c+%7e32%3b+ii)+Bangladesh%2c+%7e25%3b+iii)+-Vietn&u=a1aHR0cHM6Ly9jb21tZXJjZS5nb3YuaW4vdHJhZGUtc3RhdGlzdGljcy8&ntb=1
16. V.K Gondalia, K.B. Kathiria, K.S Jadav, Ganga Devi, Rachana Bansal, A.S. Shaikh, Jignesh Macwan, Growth and Prospects of Export of Major Seed Spices from India, Nutrition and Dietetics, Directorate of Research, March 2022
17. Chandrasekar Kandaswamy, Sreekanth Anandaram, S. I. Davis Presley, and Ahammed T. P. Shabeer, Comparative evaluation of multi-residue methods for analysis of pesticide residues in black pepper by gas chromatography tandem mass spectrometry: critical evaluation of matrix co-extractives and method validation, ., 2021 Mar; 58(3): 911–920
18. Fernanda Volpatto, Arci Dirceu Wastowski, Gabrieli Bernardi, Osmar Prestes, Evaluation of QuEChERS Sample Preparation and Gas Chromatography Coupled to Mass Spectrometry for the Determination of Pesticide Residues in Grapes, Journal of the Brazilian Chemical Society, 2016 September, 27(9) DOI:10.5935/0103-5053.20160032
19. Sample preparation techniques for suspect and non-target screening of emerging contaminants, Parvaneh Hajeb, Linyan Zhu, Rossana Bossi, Katrin Vorkamp, Aarhus University, Department of Environmental Science, Roskilde, Denmark, Chemosphere, Volume

287, Part 3, January 2022, 132306
20. QuEChERS Procedure for Multiresidue Pesticide, https://www.waters.com/webassets/cms/library/docs/720003643en.pdf
21. Styliani E. Romniou, Konstantina Nana, Marilena Dasenaki, Efstratios Komaitis, and Charalampos Proestos, Development and Validation of Pesticide Residues Determination Method in Fruits and Vegetables through Liquid and Gas Chromatography Tandem Mass Spectrometry (LC-MS/MS and GC-MS/MS), Employing Modified QuEChERS Method and a Centrifugal Vacuum Concentrator
22. Analytical Quality Control and Method Validation procedures for pesticide residues in food and feed SANTE /12682/2019
23. Mahesh Kumar Saini, Samsul Alam, Sudeep Mishra, Lalit Kumar Thakur, January 2016, Method Development and Validation of Multiclass Pesticide Residues and Metabolites in Wheat by GC-ECD and GC-MS, Asian Journal of Research in Chemistry , 9 (1):13, 2016 January , DOI: 10.5958/0974-4150.2016.00003.
24. Hirokazu Saegusa, Hiroshi Nomura, Masaki Takao, Takashi Hamaguchi, Masaru Yoshida, and Yuzo Kodama, Development and validation of an analysis method for pesticide residues by gas chromatography–tandem mass spectrometry in Daikenchuto, J Nat Med. 2021; 75(2): 344–360., 2021 Jan 3. doi: 10.1007/s11418-020-01473-y.
25. Maria Constantinou, Despo Louca-Christodoulou, Agapios Agapiou, Method validation for the determination of 314 pesticide residues using tandem MS systems (GC–MS/MS and LC-MS/MS) in raisins: Focus on risk exposure assessment and respective processing factors in real samples (a pilot survey), Food Chemistry, Volume 360, 30 October 2021, 129964, https://doi.org/10.1016/j.foodchem.2021.129964
26. Geeta Mathur, Priyanka Bhardwaj, Nidhi K, Manjeet A, R. K. Khandal, Notable Trends of Quality of Spices: Need for Persistence Studies on Residues and Harmonization, Journal of Advanced Research in Food Science and Nutrition Volume 6, Issue 1 - 2023, Pg. No. 1-9
27. Rushali Girame, T.P. Ahammed Shabeer, Bappa Gh osh, Sandip Hingmire, Ramesh Natarajan, Pradyu mn Narayan Dubey, Multi-residue method validation and safety evaluation of pesticide residues in seed spices cumin (Cuminum cyminum) and coriander (Coriandrum sativum) by gas chromatography tandem mass spectrometry (GC–MS/MS), 16 April 2022, Volume 374, 131782, https://doi.org/10.1016/j.foodchem.2021.13178
28. Bożena Łozowicka, Ewa Rutkowska, and Magdalena Jankowska, Influence of QuEChERS modifications on recovery and matrix effect during the multi-residue pesticide analysis in soil by GC/MS/MS and GC/ECD/NPD, Environmental Science and Pollution Research International. 2017 Jan 16; 24(8): 7124–7138. doi: 10.1007/s11356-016-8334-1
29. P. Parrilla Vázquez, C. Ferrer, M.J. Martínez Bueno, A.R. Fernández-Alba, Pesticide residues in spices and herbs: Sample preparation methods and determination by chromatographic techniques, TrAC Trends in Analytical Chemistry, Volume 115, June 2019, Pages 13-22, https://doi.org/10.1016/j.trac.2019.03.022
30. Ewa Rutkowska, Bożena Łozowicka, Piotr Kaczyński, Three approaches to minimize matrix effects in residue analysis of multiclass pesticides in dried complex matrices using gas chromatography tandem mass spectrometry, Food Chemistry, 1 May 2019, Volume 279, Pages 20-29