Development and method validation for determination of 54 pesticides in Okra by LC-MS/MS analysis

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**ABSTRACT**
The presence of pesticide residues in primary and derived agricultural products raises serious health concerns for consumers across the globe. The aim of the present study was to assess the level of pesticide residues in Okra in India. A multi-residue method for the quantification of fifty-four pesticides in okra is described in this work. The present study employed a modified quick, easy, cheap, effective rugged and safe (QuEChERS) extraction procedure followed by UHPLC-MS/MS (Ultra-High-Performance Liquid Chromatography coupled to Tandem Mass Spectrometry) analysis. Validation of the method was according to the guidelines given by European Union SANCO/12571/2013. The levels of validation were 10.0, 50.0 and 100 μg kg⁻¹. The following parameters such as linearity, the limit of detection (LOD) (nearer to 0.005 mg kg⁻¹) and limit of quantification (LOQ) (nearer to 0.01 mg kg⁻¹) were set to be acceptable. The trueness of the method for 54 pesticides in all Okra commodities was between 80-110% with satisfactory repeatability and within-run reproducibility except for the pesticide residues such as Thiamethoxam and Fenamidone. The measurement of uncertainty for each of the pesticide was below 50% and was estimated to be in the range of 5.37% - 10.71%, which meets the criteria established in the SANCO/12571/2013 document (European Union, 2013). This method is concluded to be applicable for the determination of pesticide residues in Okra.

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**INTRODUCTION**
Okra (*Abelmoschus esculentus*) is a popularly grown vegetable across the world. It is a herbaceous plant and is grown for the edible seed pods, which belongs to the Malvaceae family (*Khan et al.*, 2009). It is the most commonly grown crop in tropical and subtropical countries of the world, including India (*András et al.*, 2005; *Saifullah and Rabbani*, 2009). Okra is a vegetable crop that is dominated by many insect pests and hence a lot of pesticide residues have been employed by the vegetable growers for getting good quality and yield. The crop plants used as food sources treated with pesticides may retain some amounts of these residues. Hence methods for the decontamination of these crops must be introduced as these chemicals pose harmful effects on human health. They also play an important role in environmental contamination, Biodiversity loss and spoilage of natural environments (*Subhani et al.*, 2001). Established pesticide residue monitoring is present in well-developed countries. It is of utmost importance that the use of Bifenthrin and Profenofos has been increased in okra over the past years.

Food and other agricultural crops are analysed for chemical residues using various methods such as gas chromatography (GC) and liquid chromatogra-
phy (LC). The development of specific as well as sensitive methods for the determination of traces of residues, is very critical. LC has been proved to be very beneficial for the analysis of compounds with regards to the cost and procedure used for derivatization when compared to GC (Fang et al., 2012; Tadeo et al., 2010). However, with the latest technological advancements, mass spectrometry (MS) in tandem with liquid chromatography (LC-MS/MS) is favoured for both precise and accurate identification and quantification of the compounds (Fang et al., 2012). LC-MS/MS is based on triple quadrupole (OqO) and has been commonly used in the food and environmental analysis because of high sensitivity and two Multiple Reaction Monitoring (MRM) transitions are recorded (Núñez et al., 2012). Currently, MS/MS is coupled with Ultra-Fast Liquid Chromatography (UHPLC), which is known to be highly selective and sensitive.

Sample preparation in food analysis has still become a major challenge though some latest extraction techniques used in pesticide residue analysis were developed as most of the advanced analytical techniques have several disadvantages. Need for simple and robust methods are being developed and one such method is Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS). This method has been changed and validated and more often used for broad range multi-residue pesticide analysis in food. In the present study, we report the development and validation of a method for the identification of 54 pesticide residues in Okra, aiming to meet the demands of the Central Insecticide Board and Registration Committee in India and Food Safety and Standards Authority of India (CIBRC & FSSAI). Okra samples were processed by a modified (QuEChERS) extraction procedure with a clean-up step and were analysed by LC-MS/MS. The method validation was carried out by following the guidelines provided by the European Union SANCO/12571/2013 (Union, 2013).

MATERIALS AND METHODS

Reagents and Chemicals

Analytical grade reagents were employed in this study. Acetonitrile and glacial acetic acid (HPLC-grade) purchased from Merck (India). Methanol was procured from J.T Baker, magnesium sulphate was purchased from Agilent, while sodium acetate purchased from Merck (India) and ammonium acetate was purchased from (Sigma). Formic acid was purchased from Merck (Xalostoc, Mexico). Primary & secondary amine sorbent (PSA) and Graphitised carbon block (GCB) purchased from Agilent, ultra-pure water and HPLC water procured from Merck (Xalostoc, Mexico). The stock solutions were prepared at 1000 ppm in an appropriate solvent, stored at -20 ± 2 °C in a deep freezer. From the above stock solution, the working standards were prepared.

Instrumental conditions

Chromatography

The chromatographic analyses were performed using an UHPLC, which is equipped with a Quaternary pump (LC20AD), an autosampler (SIL20AC) and a column oven (CTO20A). Shim-pack XR-ODS III column (75 × 2 mm, 1.6 μm) employed for the separation of the pesticide residues. The mobile phase employed for separation contained 10 mM ammonium acetate in water (Mobile phase A) and 10 mM ammonium acetate in methanol (Mobile phase B) at 0.4 mL/min flow rate. The gradient elution was programmed as: A (65%–B (35%) (4 min), A (40%–B (60%) (9 min), A (30%–B (70%) (5 min), A (10%–B (90%) (2.0 min), and A (65%–B (35%) (2 min). The total run time programmed was 22 min and the volume injected was 5 μl with the column oven temperature at 40 °C.

Mass spectrometry

Mass spectrophotometry (8040 Triple Quadrupole MS, Lab ware solutions, Schimadzu, Japan) with following instrumental conditions, Desolvation gas temperature: 250 °C; Heat block temperature: 300 °C; Drying gas flow – 15 L/min; Nebulizer gas (N2) flow- 2 L/min; Dwell time – 10 msec⁻¹; Interface voltage – 4.5-5.0 kv. At these conditions, the retention times of all compounds and the Multiple Reaction Monitoring (MRM) transitions used for the quantitative and qualitative estimation are presented in Tables 1 and 2. Linearity was calculated by determining the correlation coefficient (r²) from calibration curve obtained based on six different concentrations (10 ppb, 20 ppb, 40 ppb, 60 ppb, 80 ppb, 100 ppb) of pesticide residues. Recovery was validated by fortifying the untreated okra vegetable samples with standard solutions of the mix of 54 compounds. The LOD (limit of detection) for 54 compounds was nearer to 0.005 mg/kg and the limit of quantification (LOQ) being nearer to 0.01 mg/kg.

Preparation of sample

Sample extraction

The modified QuEChERS extraction was applied for the sample (clean up step with the addition of GCB (Graphitised Carbon Black) and without the addition of GCB) (Madureira et al., 2012). Magnesium sulfate was added for removing residual water and PSA (Primary Secondary amine Sorbent) was employed for sugar and carbohydrates removal.
### Table 1: Retention time and MS/MS conditions for each compound

| Compound name                  | R.T(min) | Precursor | Quanti-fierion | Collision energy | Qualifier | Collision energy |
|--------------------------------|----------|-----------|----------------|------------------|-----------|------------------|
| Demeton-s-Methylsulfone        | 1.6      | 263       | 168.9          | 30               | 109       | 19               |
| Buprofezin                     | 13.1     | 306.2     | 201            | 30               | 116.1     | 20               |
| Dimethoate                     | 3.0      | 230       | 198.95         | 19               | 125       | 22               |
| Carbendazim                    | 4.1      | 192       | 159.95         | 28               | 131.95    | 23               |
| Tricyclozole                   | 4.9      | 190       | 135.95         | 23               | 162.95    | 30               |
| Emamectin benzoate             | 13.9     | 886.7     | 158.0          | 21               | 126.0     | 25               |
| Hexaconazole                   | 15.3     | 314.1     | 70.05          | 28               | 92        | 16               |
| Chlorpyriphos                  | 16.9     | 351.9     | 199.8          | 17               | 199.8     | 19               |
| Phorate                        | 15.4     | 261       | 75.05          | 30               | 47.05     | 18               |
| Chlorfenviphos                 | 15.2     | 360.9     | 155            | 29               | 98.9      | 17               |
| Ethion                         | 16.8     | 384.9     | 199            | 19               | 142.95    | 24               |
| Spinosad-d                     | 17.9     | 746.4     | 142.15         | 25               | 98.15     | 17               |
| Allethrin                      | 16.7     | 303.2     | 135            | 25               | 123.05    | 21               |
| Cypermethrin                   | 17.5     | 433.1     | 190.95         | 18               | 127       | 22               |
| Pendimethalin                  | 17.0     | 282.1     | 212            | 21               | 43.15     | 16               |
| Fenpropathrin                  | 16.9     | 349.9     | 96.9           | 16               | 125       | 21               |
| Spinosad-a                     | 17.5     | 732.4     | 142.1          | 24               | 99.1      | 17               |
| Bifenthrin                     | 18.5     | 440.2     | 181.05         | 18               | 166       | 30               |
| Abamectin                      | 17.9     | 890.4     | 305.2          | 28               | 501       | 19               |
| Profenophos                    | 16.3     | 372.9     | 302.8          | 19               | 299       | 29               |
| Thiamethoxam                   | 1.8      | 293.5     | 211.1          | 18               | 192       | 25               |
| Imidacloprid                   | 2.3      | 256       | 209.05         | 20               | 175.1     | 30               |
| Acetamiprid                    | 3.0      | 223       | 126            | 21               | 99        | 16               |
| Methamidophos                  | 1.3      | 142       | 94             | 16               | 125       | 23               |
| Acephate                       | 1.3      | 184       | 143.0          | 27               | 49.15     | 20               |
| Methomyl                       | 1.7      | 163       | 88.05          | 15               | 106.1     | 19               |
| Thiocloprid                    | 4.1      | 253       | 125.9          | 21               | 90.05     | 15               |
| Monocrotophos                  | 3.0      | 224       | 127.0          | 21               | 193       | 19               |

This procedure has been validated (in-house) to satisfy the European Union SANCO/12571/2013 guidelines (Union, 2013).

The okra samples without any pesticide interference were employed as blanks for validation experiments. Two extraction procedures were employed for the extraction of Blank okra samples, 1st without the addition of GCB in clean up step and another with GCB in clean-up step. A representative sample was homogenized and ~15.0 g of the sample has been transferred into a 50 ml tarsion tube and appropriate amounts of multi pesticide mix standards were added. Subsequently, 30 ml of acetonitrile (v/v) was added and the mixture was homogenised with homogenizer and vortexed for 1 minute. Then 3.0 g of anhydrous sodium chloride was added and vortexed for 30 seconds and centrifuged at 2500 rpm for 10 minutes. The major use of sodium chloride was it would separate the organic layer and aqueous layer. The organic layer (~16 ml supernatant) was transferred to 50 ml of tarsion tube supplemented with anhydrous sodium sulphate (9.0 g) and thoroughly shaken, vortexed for 1 min. The use of sodium sulphate was it would absorb the moisture. The supernatant (8 ml) obtained was transferred to a 15 ml tarsion tube containing 1500 mg of magnesium sulphate and 400 mg of PSA and 7.5 mg of GCB. The mixture was vortexed and centrifuged for 10 min at 2500 rpm. An aliquot of 1 ml supernatant eventually was transferred to a Ria vial then evaporated at 45 C in a turbo evaporator. The residue was reconstituted with the mobile phase (1ml), filtered and injected into the LC-MS/MS...
Table 2: Retention time and MS/MS conditions for each compound (Continued from Table 1)

| Compound name | R.T(min) | Precursor | Quantifier | Collision energy | Qualifier | Collision energy |
|---------------|----------|-----------|------------|------------------|-----------|------------------|
| Simazine      | 7.9      | 202.1     | 124.0      | 26               | 68.05     | 27               |
| Carbofuran    | 7.9      | 222.1     | 165.1      | 29               | 123.05    | 20               |
| Dichlorvas    | 7.9      | 222       | 165        | 29               | 123       | 22               |
| Thiodicarb    | 9.8      | 355       | 88         | 14               | 108.1     | 18               |
| Phosphomidan  | 7.1      | 300       | 174.0      | 30               | 127       | 20               |
| Malaxon       | 8.5      | 315       | 127.0      | 16               | 99.05     | 21               |
| Carbaryl      | 8.7      | 202.1     | 145        | 25               | 127.1     | 21               |
| Atrazine      | 10.0     | 216.1     | 174.1      | 30               | 96.15     | 15               |
| Metalaxyl     | 10.4     | 280.1     | 220.0      | 22               | 192.1     | 18               |
| Fenamidone    | 12.2     | 312.1     | 236        | 16               | 90.05     | 23               |
| Nitenpyram    | 13.4     | 271.1     | 125.9      | 31               | 224.9     | 25               |
| Myclobutanil  | 13.2     | 289.1     | 70         | 29               | 124.95    | 19               |
| Malathion     | 12.9     | 331       | 127        | 17               | 99        | 22               |
| Spirotetramate| 14.0     | 374.2     | 302.1      | 19               | 216.05    | 20               |
| Pyrifroxyfen  | 13.8     | 320.9     | 92.0       | 21               | 160.05    | 15               |
| Alachlor      | 13.9     | 270.1     | 238.1      | 15               | 162.15    | 18               |
| Phosalone     | 15.0     | 367.9     | 125        | 21               | 182       | 17               |
| Penconazole   | 14.8     | 284.1     | 70         | 28               | 158.9     | 26               |
| Quinolophos   | 14.7     | 299       | 147.1      | 28               | 163       | 25               |
| Anilophos     | 15.0     | 369.9     | 198.9      | 21               | 125       | 19               |
| Diazonin      | 15.2     | 305.1     | 169.05     | 29               | 153.1     | 25               |
| Tebuconazole  | 14.9     | 308.1     | 70.05      | 27               | 125.05    | 22               |
| Indoxycarb    | 16.0     | 527.9     | 203        | 30               | 293       | 18               |
| Trifloxy      | 16.0     | 409       | 186.05     | 18               | 206.05    | 20               |
| Spiromesifen  | 17.1     | 371       | 273.1      | 17               | 255.1     | 25               |
| Azinophos Ethyl | 11.2   | 346       | 77         | 19               | 132       | 21               |

Method validation

Selectivity and Linearity curves

The method selectivity was assessed by blank solution injections. It has been observed that the method is free of pesticide interferences, as is indicated by the absence of the signal at retention times of the target compounds. To minimise the effect of matrix, Matrix-matched calibration (MMC) was used. Analytical MMC curves were constructed using blank okra extracts with the appropriate quantity of pesticide mix standard at different levels such as 10, 20, 40, 60, 80 and 100 μg/kg. For simultaneous quantification and identification purposes, two MRM transitions for each analyte were used to avoid false negatives at trace pesticide levels. The data obtained were analysed using Lab Solution Software. The appropriate dilution by a dilution factor is necessary for the compounds for which the MRL is above the working range and such dilution is applied for the uncertainty calculation.

LOD, LOQ and measurement of uncertainty

The LOD and LOQ were experimentally identified by spiked blank okra samples with standards at six levels starting from lowest at which all the analytes are detected. A calibration curve was plotted and from the calibration curve LOD & LOQ values were determined using the following formula (LOD=3×SD/Slope, LOQ=10×SD/Slope). The LOD was the lowest concentration of analyte in a sample that can be detected and not quantified. The LOQ is the lowest spike level meeting the method performance criteria true-ness and precision (70-120% and RSD ≤ 15%, respectively). Measure-
Table 3: Method validation parameters for each of the analyte

| Compound Name             | Correlation coefficient | Average recovery % with %RSD | Measurement of Uncertainty | LOD  | LOQ  |
|---------------------------|-------------------------|-------------------------------|---------------------------|------|------|
|                           |                         | 10μg/kg                       | 100μg/kg                  |      |      |
|                           |                         | 7.8                           | 6.12                      | 1.93 | 6.44 |
| Demeton-s-Methylsulphone  | 0.998                   | 91.53(7.1)                    | 100.90(3.9)               |      |      |
| Buprofezin                | 0.996                   | 99.97(7.9)                    | 90.56(6.8)                | 8.3  | 7.58 |
| Dimethoate                | 0.999                   | 94.74(9.4)                    | 95.77(7.1)                | 9.2  | 7.8  |
| Carbendazim               | 0.999                   | 94.99(9.2)                    | 89.72(4.6)                | 9.1  | 6.42 |
| Tricyclozole              | 0.999                   | 93.40(5.7)                    | 91.58(6.6)                | 7    | 7.5  |
| Emamectin benzoate        | 0.995                   | 96.01(6.4)                    | 97.91(4.1)                | 7.4  | 6.18 |
| Hexaconazole              | 0.996                   | 96.01(6.9)                    | 99.26(7.2)                | 7.6  | 7.87 |
| Chlorpyriphos             | 0.996                   | 101.74(7.7)                   | 88.39(5.0)                | 8.1  | 6.6  |
| Phorate                   | 0.995                   | 93.55(8.4)                    | 109.33(2.9)               | 8.6  | 5.71 |
| Chlorfenviphos            | 0.997                   | 91.34(8.5)                    | 95.35(8.6)                | 8.6  | 8.73 |
| Ethion                    | 0.995                   | 100.86(8.9)                   | 102.95(3.7)               | 8.98 | 6.02 |
| Spinosad-d                | 0.998                   | 99.47(8.8)                    | 94.70(4.1)                | 8.91 | 6.19 |
| Allethrin                 | 0.995                   | 93.22(6.5)                    | 104.31(3.4)               | 7.48 | 5.9  |
| Cypermethrin              | 0.999                   | 91.78(8.0)                    | 92.9(8.8)                 | 8.35 | 8.84 |
| Pendimethalin             | 0.998                   | 94.64(9.1)                    | 107.06(4.2)               | 10.43| 6.22 |
| Fenpropathrin             | 0.995                   | 97.04(7.1)                    | 98.39(5.9)                | 8.19 | 7.08 |
| Spinosad-a                | 0.995                   | 93.60(9.6)                    | 92.09(5.7)                | 9.41 | 6.96 |
| Bifenthrin                | 0.995                   | 100.83(8.6)                   | 88.34(4.7)                | 8.79 | 6.47 |
| Abamectin                 | 0.999                   | 96.89(10.0)                   | 97.87(7.1)                | 9.75 | 7.81 |
| Profenophos               | 0.995                   | 92.03(6.9)                    | 95.66(8.8)                | 7.69 | 8.85 |
| Thiamethoxam              | 0.997                   | 111.71(3.3)                   | 113.61(2.6)               | 5.87 | 5.62 |
| Imidacloprid              | 0.997                   | 96.99(7.7)                    | 103.90(4.7)               | 8.21 | 6.45 |
| Acetamiprid               | 0.999                   | 97.67(6.2)                    | 90.49(5.4)                | 8.59 | 6.8  |
| Methamidophos             | 0.998                   | 93.99(7.3)                    | 102.41(4.2)               | 7.91 | 6.23 |
| Acephate                  | 0.998                   | 93.95(11.4)                   | 97.10(7.2)                | 10.71| 7.87 |
| Methomyl                  | 0.996                   | 97.96(8.0)                    | 89.03(5.7)                | 8.41 | 6.98 |
| Thiocloprid               | 0.999                   | 94.97(9.4)                    | 98.59(8.0)                | 9.33 | 8.32 |
| Monocrotophos             | 0.996                   | 88.99(8.1)                    | 101.38(6.1)               | 8.45 | 7.21 |

Trueness and Precision

The method trueness has been determined using the values obtained from the recovery assay. The samples were spiked with all the analytes at three concentrations – 10.0, 50.0 and 100.0 μg/kg (n=6 at each concentration) and analysed for three different days by two analysts. Recovery of the analytes was calculated by using the true value and analysed value. The data obtained is used for determining the precision and uncertainty measurement (MU). Relative Standard Deviation (RSD) was estimated using the samples (n=6) that were analysed on the same day at each concentration. The precision was expressed as relative standard deviation (RSD) and is determined with replication data (n=18) of 3 different days at each concentration level and the results obtained are presented in Tables 3 and 4.

RESULTS AND DISCUSSION

The chromatogram showing the total ions (TIC) is given in Figure 1. To get the chromatogram, blank extracts Okra samples were fortified with all the pesticide mix at 10.0 ppb and the more intense
Table 4: Method validation parameters for each of the analyte (Continued from Table 3)

| Compound Name | Correlation coefficient | Average recovery percent with %RSD | Measurement of Uncertainty | LOD | LOQ |
|---------------|-------------------------|------------------------------------|-----------------------------|-----|-----|
|               |                         | 10 µg/kg | 100 µg/kg | 10 µg/kg | 100 µg/kg |
| Simazine      | 0.997                   | 97.61(8.4) | 97.56(9.8) | 8.65 | 9.54 | 2.7 | 9   |
| Carbofuran    | 0.998                   | 108.79(7.0) | 96.48(7.2) | 10.2 | 7.86 | 2.13 | 7.1 |
| Dichlorvas    | 0.999                   | 86.29(7.0) | 95.39(8.2) | 9.93 | 8.5 | 2.35 | 7.1 |
| Thiodicarb    | 0.998                   | 89.29(6.6) | 93.23(8.5) | 7.51 | 8.7 | 2.73 | 9.1 |
| Phosphomidan  | 0.999                   | 105.39(4.6) | 85.71(4.2) | 6.46 | 6.22 | 2.28 | 7.61 |
| Malaxon       | 0.997                   | 95.93(11.1) | 98.31(4.4) | 10.46 | 6.3 | 2.5 | 8.34 |
| Carbaryl      | 0.999                   | 96.38(5.9) | 101.40(5.8) | 10.79 | 7.02 | 2.51 | 8.37 |
| Atrazine      | 0.999                   | 94.24(5.7) | 89.02(6.9) | 7 | 7.69 | 2.67 | 8.92 |
| Metalaxyl     | 0.998                   | 97.46(3.7) | 99.23(3.8) | 6.04 | 6.04 | 2.81 | 9.38 |
| Fenamidone    | 0.998                   | 116.66(1.6) | 112.67(2.3) | 5.37 | 5.53 | 2.8 | 9.34 |
| Nitenpyram    | 995                     | 96.08(6.3) | 87.84(4.9) | 7.33 | 6.56 | 2.52 | 8.43 |
| Myclobutanil  | 0.998                   | 95.99(7.3) | 104.10(6.8) | 7.92 | 7.6 | 2.48 | 8.27 |
| Malathion     | 0.999                   | 99.55(6.8) | 86.68(4.0) | 7.66 | 6.12 | 2.5 | 8.36 |
| Spirotetramate| 0.999                   | 92.22(4.8) | 96.82(8.1) | 8.24 | 8.44 | 2.23 | 7.44 |
| Pyrifloxyfen  | 0.999                   | 91.91(8.9) | 104.30(4.5) | 8.95 | 6.39 | 2.36 | 7.89 |
| Alachlor      | 0.998                   | 95.04(9.4) | 92.57(5.8) | 9.29 | 7.03 | 1.58 | 5.26 |
| Phosalone     | 0.998                   | 94.79(9.3) | 86.56(3.9) | 9.26 | 6.08 | 2.78 | 9.27 |
| Penconazole   | 0.998                   | 94.54(8.4) | 97.57(6.7) | 8.64 | 7.55 | 2.87 | 9.59 |
| Quinophos     | 0.996                   | 95.30(9.1) | 86.35(4.9) | 9.08 | 6.56 | 2.25 | 7.52 |
| Anilopshes    | 0.997                   | 92.82(10.2) | 94.25(6.7) | 9.88 | 7.54 | 2.58 | 8.6 |
| Diazonin      | 0.995                   | 97.91(10.1) | 106.04(6.7) | 9.78 | 7.55 | 2.34 | 7.83 |
| Tebuconazole  | 0.998                   | 99.28(9.8) | 96.33(3.5) | 9.56 | 5.95 | 2.34 | 7.8 |
| Indoxycarb    | 0.997                   | 91.79(7.9) | 103.95(4.0) | 8.35 | 6.14 | 2.43 | 8.11 |
| Triflouxystrobin| 0.997            | 93.76(9.2) | 84.62(3.3) | 9.17 | 5.85 | 2.65 | 8.86 |
| Spiromesifen  | 0.998                   | 95.17(9.7) | 108.55(4.3) | 9.51 | 6.28 | 1.47 | 4.92 |
| Azinophos-Ethyl| 0.995               | 85.70(4.7) | 87.08(4.1) | 6.09 | 6.45 | 3.51 | 9.85 |

MRM transition for each compound was picked up in Tables 1 and 2. The precursor (parent) ion and the two MRM transitions (quantification and identification ion) should necessarily be present according to SANCO/12571/2013 guidelines (Union, 2013). The ratio between qualifier ion transitions of the sample and the standard should be less than 30%.

**Extraction procedure**

Then QuEChERS procedure with the addition of GCB was employed for the extraction of pesticides in okra as the use of GCB reduce noise ratio, system contamination and good recovery percentages (Anastassiadis et al., 2003; Lehotay et al., 2005).

**Method validation**

The method validation was carried out by following the guidelines given by the European Union SANCO/12571/2013 (Union, 2013). LOD and LOQ are represented in Tables 3 and 4. It has been observed that the LODs and LOQs were nearer to 5.0 and 10 µg kg, respectively.

The trueness and precision were estimated, which were shown in Tables 3 and 4. The analysis of the results showed that recoveries were in acceptable range (80-110%) except Fenamidone and Thiamethoxam. As can be seen in Tables 3 and 4, almost all the results indicate that the % RSD is less than 15% for all the levels of fortification. The main uncertainty sources of the method,

(1) Due to Sample (Repeatability Measurement)
(2) Electronic balance
(3) Purity of standard,
(4) Glassware
(5) Standard Curve
(6) Micro Pipette
As it was depicted in Tables 3 and 4, that MU for each of the pesticide was below 50% and was estimated to be in the range of 5.37% - 10.71%. The results are in accordance with the established criteria in SANCO/12571/2013 document (Union, 2013).

Recovery in the range of 80-110% was obtained for all the compounds except for two compounds, Thiamethoxam and Fenamidone, showing recovery above 110% at three concentration levels. An intermediate precision % RSD of all the compounds showing below 15% at three concentration levels and measurement uncertainty of all the compounds were considered satisfactory.

CONCLUSIONS

A rapid and multi-residue method for the determination of 54 pesticides in okra was developed and presented in this paper. The method has been proved to be simple and provided good validation parameters (linearity, limits of detection, quantification and precision). The values of uncertainty obtained for each compound were below 50% at all levels of fortification, which meets the SANCO/12571/2013 guidelines. The developed method stands best in determining the target pesticides in real samples and can be applied in routine analysis of pesticides.

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