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# A quick method for surveillance of 59 pesticide residues in fruits and vegetables using rapid three-dimensional gas chromatography (GC/MSD/μ-ECD/FPD) and LC/MS-MS

Gouri Satpathy[a], Yogesh Kumar Tyagi[a,*] and Rajinder Kumar Gupta[b]

[a] *University School of Basic and Applied Sciences, Guru Gobind Singh Indraprastha University, Sector 16 C, Dwarka, Delhi-110075, India*
[b] *University School of Biotechnology, Guru Gobind Singh Indraprastha University, Sector 16 C, Dwarka, Delhi-110075, India*

**Corresponding author at: University School of Basic and Applied Sciences, Guru Gobind Singh Indraprastha University, Sector 16 C, Dwarka, Delhi-110075, India. Tel.: +91.11.25352482; fax: +91.98.99562482. E-mail address: drytyagi@gmail.com (Y.K. Tyagi).*

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ABSTRACT

This article describes a simple, quick and inexpensive method for determination of pesticides in fruits and vegetables. The method, known as the quick, easy, cheap, effective, rugged and safe (QuEChERS) method for pesticide residues, involves the extraction and simultaneous liquid-liquid partitioning formed by adding anhydrous magnesium sulfate ($MgSO_4$) plus sodium acetate (NaAc) followed by a simple cleanup step known as dispersive solid-phase extraction (dSPE). The extracts were analyzed by three-dimensional gas chromatography GC/MSD/μ-ECD/FPD in trace ion mode and liquid chromatography/tandem mass spectrometry (LC/MS-MS). Method sensitivity, linearity, repeatability and reproducibility, accuracy, matrix effects, and overall uncertainties have been studied for method validation according to the international norm ISO/IEC: 17025:2005 for both techniques. Identification, quantification and reporting with Total and Extracted ion chromatograms, μECD and DFPD were facilitated to a great extent by Deconvolution Reporting Software (DRS) for GC and Mass hunter software for LC. For all compounds LODs were 0.001 to 0.01 mg/kg and LOQs were 0.005 to 0.020 mg/kg. Correlation coefficients of the calibration curves were >0.991. To validate the effects of matrices, repeatability, reproducibility, recovery and overall uncertainty were calculated for twenty-four matrices at 0.020, 0.050 and 0.500 mg/kg. Recovery ranged between 75-107 % with RSD <17 % for repeatability and intermediate precision and UM of ± 13-22 %.

## 1. Introduction

With the advent of High Yielding Varieties (HYV) that marked the Green Revolution and the ever increasing demand on the agricultural sector in India, the application of pesticides to various crops has increased manifold. Pesticides are a chemically diverse group of compounds which enhance harvest productivity by controlling pests. The use of pesticides enhances the antioxidant potential in medicinal plants which is proven to be beneficial for farmers [1]. However, some pesticides are known to cause birth defects and adversely affect the functioning of central nervous system, respiratory system and endocrine system. In addition, long term exposure beyond tolerance limits to pesticides (which are highly toxic and probable carcinogens), is reported to induce cancer [2,3].

Considering the lethal effects of pesticides on human health, in India, Prevention Food Adulteration Act (PFA) [4] sets the Maximum Residual Limit (MRL) for various pesticide residues for different commodities.

Multi residues analysis of pesticides in fruits, vegetables and other foods is a primary function of several regulatory, industrial and contract laboratories throughout the world. It is estimated that >200,000 food samples are analyzed world-wide each year for pesticide residues to meet a variety of purposes [5]. Once analytical quality requirements have been met to suit the need for any particular analysis, all purposes for analysis favor practical benefits (high sample throughput, ruggedness, ease-of-use, low cost and labor, minimal solvent usage and waste generation, occupational and environmental friendliness, small space requirements and few material and glassware needs). A number of analytical methods designed to determine multiple pesticide residues have been developed [6-8]. In 2003, the QuEChERS method for pesticide residue analysis was introduced [9], which provides high quality results in a fast, easy and inexpensive approach in the area of sample preparation. Follow-up studies have further validated the method for >200 pesticides [10], improved results for the remaining few problematic analytes [11], and tested it in fat-containing matrices [12].

Simultaneous determination of numerous pesticides in different food matrices, as listed in The Pesticide Manual [13], with a single chromatographic is not possible, thereby requiring the application of both GC and HPLC. Out of the 59 studied pesticides, 9 were analyzed by LC-MS-MS [5] and the remaining were amenable to capillary GC analysis with MS including the other classical selective detection methods (μECD and DFPD) to analyze many classes of pesticides in a single run. It has already been proved that mass spectrometry (MS) is capable of identifying an analyte by full scan library match or multiple target and qualifier ion ratios from selected ion monitoring (SIM) [14]. However, MS sometimes lacks the selectivity to find target analyte spectra which are sometimes


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overwhelmed by similar ions contributed from the co-extractives in the matrix.

For instance, many laboratories screen food samples for semi volatile pesticides using the ECD or ELCD (or XSD) for organ halogen, FPD or pulsed FPD (PFPD) for organophosphorous, and NPD for nitrogen containing targets [15,16]. Although these three methods provide excellent selectivity and sensitivity, they lack the capability of identification. In most of these procedures, multiple injections are needed to identify hundreds of compounds at parts-per-billion (ppb) levels. To improve the efficiency and increase the productivity of screening for all of these pesticides, the challenge is to reduce the analysis time taken by GC-MS or the combination of GC and GC-MS. Therefore the compromise and the typical approach is to use selective GC detectors (ECD and DFPD) to flag potential target analytes and use MS SIM for confirmation in a single injection. Thus, to overcome these challenges, new hardware and software tools, including GCMS; capillary flow three-way splitter, back flush and trace ion detection [17], retention time locking [18], programmable temperature vaporizer injector (PTV) [19], Agilent HP-5MSi capillary column (15 m) and Deconvolution reporting software (DRS) [20,21] were used. In this method, the splitter allows multiple GC as well as MS signals to be acquired from a single injection for productivity gains (from three injections down to one). Agilent HP-5MSi capillary column reduces the analysis time; for instance, the retention time of parathion- methyl (used as the reference) in the prevalent method with a column of 30 m length was 16.569 min and in the proposed method is 7.170 min [20]. RTL [22] allows users to screen environmental and food samples for 926 pesticides and endocrine disruptors without the need of having standards at hand. It also reproduces the retention times in long term in contempt of system maintenance or other perturbations by adjusting the carrier gas flow. Trace ion detection minimizes noise in the signal and DRS separates target analyte ions from matrix background ions [23].

In the present study, a method employing GC with MSD-µECD-DFPD and LC-MS/MS detection for the separation, identification and quantification of 59 widely used pesticides in 24 fruits and vegetables was developed and validated. Finally, the method was applied for monitoring these pesticides in 403 commercial samples collected from the local markets.

## 2. Experimental

### *2.1. Materials (samples, chemicals, reagents and apparatus)*

In the multi-class and multi-residues analysis of pesticides in fruits and vegetables, twenty-four commodities of three different groups (Bottle gourd, French beans, Ridge gourd, Egg plant, Okra, Turnip, Radish, Mint, Cauliflower, Cabbage, Coriander leaves, Capsicum, Cucumber (non-starchy green fruits and vegetables), Banana, Papaya, Potato, Peas, Pomegranate, Pear (starchy/sweet fruits and vegetables), Grapes, Tomato, Plum, Apple and Orange (acidic and low acidic fruits and vegetables) were obtained from APMC (Agricultural Produce Marketing Committee), Azadpur, Delhi, India. For the homebuilt pesticide quantitation database, the CRM of pesticides >98% pure (2,4 D, Aldicarb, Aldrin, cypermethrin I & II, α-Endosulfan, α-HCH, Benomyl, β-HCH, Butachlor, Captafol, Carbaryl, Carbendazim, Chlorbenzilate, permethrin I & II, δ-HCH, Dichlorovos, Dieldrin, Dimethoate, Diuron, β-Endosulfan, Endosulfan sulphate, Fenvalerate, Esfenvalerate, Ethion, Fenthion, Heptachlor, Imidaclopride, Isoproturon, Lindane, Malathion, Methyl-parathion, OP-DDE, Paraquat-dichloride, Parathion, Permethrin I & II, Fenitrothion, Phorate, Phosalone, OP-DDD, PP-DDD, PP-DDE, Thiometon, Chlrofenvinfos-α, Chlrofenvinfos-β, Captan, Omethoate, Triadimefon, Chlorthalonil, Quinalphos, Jodfenphos, Profenofos, Endrin, Triazophos, Chlordane I & II, Chlorpyriphos), which are commonly used by local farmers in cultivation were procured from Chemco ( Chemo International, USA), Sigma-Aldrich (Sigma-Aldrich, USA) and AccuStandard (AccuStandard, USA). The standard stock solutions (1000 ppm) were prepared in ethyl acetate for GC analysis and acetonitrile for LC analysis and stored at 4 °C. All the solvents used were HPLC grade. Acetone, acetonitrile and ethyl acetate were purchased from RFCL, Delhi, India. Other chemicals: anhydrous magnesium sulphate, PSA (Primary Secondary Amine) and graphitized carbon black sorbent from Agilent Technology (LCGC, India) and acetic acid and sodium acetate were procured from Merck, India. Apparatus: Food processor homogenizer (Phillips India Ltd, Delhi India), Blender (Inter science, Japan), Vortex mixture (Jain Scientific, India), Centrifuge, Sigma 2-16 K (SV Instrument, Delhi, India) and Rotary evaporator (Caterpillar, Prama Instruments, India) were used.

### *2.2. Sample preparation*

1-2 Kg of each fresh fruits and vegetable was blended and homogenized and preserved at -20 °C. The samples were extracted by quick, easy, cheap, effective, rugged and safe (QuEChERS)) method for pesticide residues [24,25]. A representative 10 g portion of previously homogenized sample was weighed in a 200 mL PTFE centrifuge tube and was spiked with standard solution of mixed pesticides in order to give 0.005 mg/kg, 0.05 mg/kg and 0.1 mg/kg concentrations in fruits and vegetables. The mixture was sonicated for 5 min. Then, 10 mL MeCN containing 1 % acetic acid was added and the tube was shaken vigorously for 1 min. After this, 1.0 g NaAc and 4 g $MgSO_4$ were added and the shaking process was repeated for 1 min. The extract was then centrifuged (3700 rpm) for 1 min. 4 mL of the supernatant (acetonitrile phase) was then transferred to a 15 mL graduated centrifuge tube containing 200 mg PSA and 600 mg $MgSO_4$, which was then shaken energetically for 20 s. Following this, the extract was centrifuged again (3700 rpm) for 1 min. Finally, an extract containing the equivalent of 1 g of sample/ml of nearly 100 % MeCN was obtained and subjected to LC analysis. For GC analysis, an aliquot (1.0 mL) of the supernatant was evaporated, reconstituted with ethyl acetate (0.5 mL) and subjected to analysis. For samples with moderate and high levels of chlorophyll and carotinoids (for example, coriander leaves, mint, tomato, capsicum, French beans), 400 mg of (1:1) PSA mixed with graphitized carbon black (GCB) was used for clean up.

### *2.3. Instrumentation*

#### *2.3.1. GC-MSD-µECD-DFPD*

Measurements were carried out on an Agilent 7890 gas chromatograph and a three-way splitter, µECD, DFPD, and 5975B mass spectrometer in trace ion detection mode. The instrument was equipped with a programmable temperature vaporizer injector (PTV) and 7683B auto sampler (Agilent) for sample introduction. For the optimization and evaluation of low-pressure gas chromatography-mass spectrometry for the analytes were separated in an Agilent HP-5MSi capillary column (5% biphenyl/ 95% dimethylsiloxane), 15 m length, 0.25 mm id, 0.25 mm film thickness [26]. RTL, as mentioned above, compensates for retention time shifts within certain frames, but to avoid the need of relocking, a 1 m pre-column of the same film and diameter was attached to the analytical column with a quartz column connector (Agilent # 5181-3396). The retention times remained unchanged, because during maintenance, instead of cutting from the beginning of the column, a new 1 m long pre-column with the same diameter and phase as the analytical column was inserted which kept the column length constant. Since column maintenance is needed more frequently when working with samples with high matrix

content, attachment of a pre-column can prove useful in saving the column for any difficult matrices involved in GC method. The exit end of the analytical column was installed into one of the four ports on the splitter using a metal ferrule. The other three ports (Supp. File, Figure S1) were connected to three detectors via restrictors (deactivated capillary tubing) of varying diameter and length to set the split ratio among the three detectors.

Restrictors were sized for 1:1:0.1 split ratio in favour of MSD, DFPD and μECD (1/10 of the flow to MSD), with similar hold-up times. The splitter used auxiliary (Aux) electronic pneumatics control (EPC) for constant pressure makeup flow. The makeup gas (Aux pressure 3) at the splitter was fixed at 3.8 psi to maintain the split ratio throughout the run. 20 μL of injections were performed by empty baffled liner (Agilent # 5183-2037) in the PTV injector at solvent vent mode by programming as 78 °C (1.5 min), ramped at 600 °C/min to 280 °C (2 min), vent time: 1.2 min, vent flow: 100 ml/min, Purge flow: 60.0 mL/min, purge time: 2.0 min. The oven temperature program was 70 °C for 1 min, programmed to 150 °C at 50 °C/min, then to 200 °C at 6 °C/min, and finally to 280 °C at 20 °C/min; it was kept at this temperature for 5 min. A post-run was carried out for 5 min at 290 °C. During the post-run, the column head pressure was lowered to 1 psi and the pressure in the back flush increased to 60 psi. During this post-run time, the column flow was reversed in order to back flush high-boiling components from the head of the column and out through the split vent of the PTV inlet. The head pressure was calculated using the RTL software so that *parathion-methyl* was eluting at a constant retention time of 7.170 min. Quadruple Mass selective detector (MSD) was used in EI mode with scan range (*m*/*z*: 40-550). The DFPD (phosphorus or sulphur mode) was set at 250 °C, transfer line 250 °C and flow of $H_2$, air and make up gas as 75, 100 and 60 mL, respectively. The μECD was used at 250 °C with makeup gas flow set at 60 mL. The auxiliary pressure was set at 3.8 psi. The dwell time was set to 25 ms. The gas saver option was turned off; MS transfer line temperature was set to 300 °C, solvent delay was 3.0 min and the ion source and quadruple temperatures were 230 and 150 °C, respectively. Trace ion detection was turned on. Screening of pesticides was performed using the DRS in combination with the RTL pesticide library and NIST'05 library [27]. Quantitation of 45 pesticides was performed using the MSD in the selected-ion monitoring (SIM) mode at *m*/*z* (Table 1) for target and qualifier ions as well as their respective selective detectors.

The peak recognition windows used in the Agilent ChemStation were set to ± 0.2 min and in AMDIS to 12 s. These values were found to be sufficiently wide enough to compensate for some RT drift, yet narrow enough to minimize the number of false positives. The minimum match factors setting in AMDIS was set to 60. This value seemed to give the least number of false positives and false negatives.

#### *2.3.2. LC-MS/MS*

Analysis was performed with an Agilent 1100 series LC system equipped with an Agilent 6460 triple quadruple mass spectrometer, a quaternary pump, an online degasser, an auto plate-sampler and a thermostatically controlled column apartment. Chromatographic separation was carried out on a $C_{18}$ column (4.6 mm × 100 mm × 5 μm, Agilent Technology) at a flow rate of 0.6 mL/min, with a two solvent mobile phase (eluent A = 10 mM ammonium acetate and 1 % acetic acid in water; eluent B = 1 % acetic acid in methanol). The eluent gradient used is described as follows: 0-3 min, 10-40 % B; 3-7 min, 40-70 % B; 7-15 min, 90 % B; 15-20 min, 90-10 % B. The sample injection volume was 20 μL. The analytical column was thermostated at 25 °C. The following instrumental parameters were used for ESI-MS/MS (Agilent Jet stream) fragmentation: gas temp, 350 °C; gas flow, 10 L/min; nebulizer, 50 psi; sheath gas temp, 400 °C; sheath gas flow, 10 L/min; nozzle voltage, 500 V and capillary, 4000 V in positive ionization mode (Table 1). The dwell time was set to 20 ms. Data were acquired by an Agilent triple quad LC-MS Mass Hunter workstation.

### *2.4. Validation and estimation of uncertainty measurement*

The limit of detection and quantification, linearity, precision (repeatability, intermediate precision and reproducibility), robustness, accuracy and specificity has been studied for method validation, according to the ISO/IEC 17025:2005 standard and ICH guideline. In order to check the efficiency of the proposed method, the experiment was carried out by fortifying the samples 24 commodities (3 groups) of fruits and vegetables] with pesticides at three different concentrations. Six replicates for each concentration were analyzed on three different occasions together with a calibration curve to perform and establish the repeatability (intra-day precision), intermediate precision (inter-day precision) and accuracy/specificity of the method.

The measurement of uncertainty is calculated as per the ISO guide to the expression of uncertainty in measurement [28] under the repeatable and reproducible conditions for 59 pesticides in 24 commodities.

## 3. Results and discussion

### *3.1. Extraction and clean up*

For the purpose of extraction, the adoption of QuEChERS method is preferred as it is currently undergoing an extensive inter laboratory trial for evaluation and validation by pesticide monitoring programs in several countries. In brief, the extraction and clean up method is a single-step buffered acetonitrile (MeCN) extraction while salting out water from the sample by using anhydrous $MgSO_4$ to induce liquid-liquid partitioning. For cleanup, a simple, inexpensive and rapid technique called dispersive solid-phase extraction (dSPE) is conducted using a combination of primary secondary amine (PSA) sorbent to remove fatty acids and GCB for removal of pigments and carotinoids among other components and anhydrous MgSO4 to reduce the remaining water in the extract. Then the extracts are concurrently analyzed by liquid and gas chromatography (LC and GC) combined with mass spectrometry (MS) or other selective detectors to determine a wide range of pesticide residues. The advantages of the method over traditional methods of analysis are: high recovery (>85%) are achieved for a wide polarity and volatility range of pesticides, including notoriously difficult analytes; high sample throughput of about 10-20 pre-weighed samples in approx. 30-40 min is possible; here solvent usage and waste is in very small quantity and no chlorinated solvents are used; a single person can perform the method without much training or technical skills and with the use of very little glassware; it is quite rugged because extract cleanup is done to remove organic acids and colour; the MeCN is added by dispenser to an unbreakable vessel that is immediately sealed, thus solvent exposure to the worker is minimal; the reagent costs in the method are very low and only a few devices are required to carry out sample preparation [12].

### *3.2. Multi-residue screening and quantification of pesticides by GC-MSD-μECD-DFPD*

The GC system employed had a back flush device placed between the end of the column and the entrance to the MSD transfer line. A small purge gas flow mixed with the column effluent and passed through the deactivated fused silica restrictor inside the transfer line and then went into the MSD source (Supp. File, Figure S2).

**Table 1**. Fortified fruits and vegetables analysed using GC-μECD (OC), FPD (OP) and GCMSD and LC-ESI-MS/MS (others) for validation[a].

| S. No. | Name | RT | CAS NO. | Mol. weight, Target ion | SIM ions | LOD | LOQ | $r^2$ | % Recovery | Intra-day precision | Inter-day precision | ±UM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **GC-MSD- μECD –DFPD** | | | | | | | | | | | | |
| 1 | Omethoate $C_5H_{12}NO_4PS$ | 2.795 | 1113-02-6 | 213/156 | 110.0, 79.0, 109.0 | 0.005 | 0.018 | 0.992 | 76-103 | 9.8 | 16.7 | 21 |
| 2 | Dichlorovos $C_4H_7Cl_2O_4P$ | 3.579 | 7786-34-7 | 224/127 | 192.0, 109.0, 151.0 | 0.007 | 0.020 | 0.993 | 79-104 | 10.1 | 16.1 | 21 |
| 3 | Monocrotophos $C_7H_{14}NO_5P$ | 5.045 | 6923-22-4 | 223/127 | 67.0, 192.0, 97.0 | 0.006 | 0.020 | 0.996 | 80-107 | 3.5 | 7.9 | 12 |
| 4 | Phorate $C_7H_{17}O_2PS_3$ | 5.698 | 298-02-2 | 260/75 | 121.1, 260.0, 97.1 | 0.005 | 0.018 | 0.997 | 77-103 | 9.1 | 15.1 | 20 |
| 5 | α-HCH $C_6H_6Cl_6$ | 5.795 | 319-84-6 | 288/182.9 | 182.9, 218.9, 216.9 | 0.002 | 0.007 | 0.998 | 79-104 | 6.5 | 15.4 | 21 |
| 6 | Thiometon $C_6H_{15}O_2PS_3$ | 5.821 | 640-15-3 | 246/88 | 125.0, 93.0, 89.0 | 0.007 | 0.020 | 0.993 | 77-103 | 3.1 | 12.1 | 19 |
| 7 | β-HCH $C_6H_6Cl_6$ | 6.157 | 319-85-7 | 288/219 | 180.9, 182.9, 108.9 | 0.002 | 0.007 | 0.995 | 95-103 | 8.8 | 16.8 | 19 |
| 8 | Dimethoate $C_5H_{12}NO_3PS_2$ | 6.347 | 60-51-5 | 229/86.9 | 125.0, 93.0, 142.9 | 0.006 | 0.020 | 0.992 | 79-107 | 3.5 | 12.5 | 18 |
| 9 | Lindane $C_6H_6Cl_6$ | 6.816 | 58-89-9 | 288/180.9 | 182.9, 108.9, 218.9 | 0.002 | 0.007 | 0.994 | 82-104 | 4.2 | 13.2 | 21 |
| 10 | δ-HCH $C_6H_6Cl_6$ | 6.952 | 319-86-8 | 288/108.9 | 182.8, 218.9, 215.9 | 0.002 | 0.007 | 0.998 | 86-102 | 7.7 | 15.7 | 20 |
| 11 | Chlorothalonil $C_8Cl_4N_2$ | 7.006 | 1897-45-6 | 264/265.8 | 263.8, 267.8, 108.9 | 0.004 | 0.017 | 0.996 | 75-98 | 4.9 | 13.9 | 19 |
| 12 | Formothion $C_6H_{12}NO_4PS_2$ | 7.027 | 2540-82-1 | 257/93 | 125.0, 126.0, 169.0 | 0.007 | 0.020 | 0.992 | 91-101 | 6.8 | 15.9 | 21 |
| 13 | Methyl Parathion $C_8H_{10}NO_5PS$ | 7.170 | 298-00-0 | 263/109 | 263.0, 124.90, 0.0 | 0.006 | 0.020 | 0.994 | 79-103 | 2.9 | 16.0 | 21 |
| 14 | Heptachlor $C_{10}H_5Cl_7$ | 7.227 | 76-44-8 | 370/100 | 271.8, 273.8, 269.8 | 0.003 | 0.010 | 0.993 | 86-101 | 9.1 | 14.5 | 20 |
| 15 | Fenitrothion $C_9H_{12}NO_5PS$ | 7.538 | 122-14-5 | 277/277 | 125.0, 109.0, 260.0 | 0.006 | 0.018 | 0.998 | 85-103 | 8.2 | 13.2 | 21 |
| 16 | Aldrin $C_{12}H_8Cl_6$ | 7.593 | 309-00-2 | 362/66 | 262.9, 264.8, 91.1 | 0.005 | 0.020 | 0.991 | 81-102 | 8.4 | 12.4 | 17 |
| 17 | Malathion $C_{10}H_{19}O_6PS_2$ | 7.613 | 121-75-5 | 330/125 | 173.0, 127.0, 92.9 | 0.006 | 0.020 | 0.994 | 89-102 | 8.9 | 16.8 | 20 |
| 18 | Fenthion $C_{10}H_{15}O_3PS_2$ | 7.717 | 55-38-9 | 278/273 | 125.0, 109.0, 169.0 | 0.010 | 0.020 | 0.996 | 94-104 | 7.8 | 15.9 | 18 |
| 19 | Parathion $C_{10}H_{14}NO_5PS$ | 7.884 | 56-38-2 | 291/290.9 | 108.9, 96.9, 138.9 | 0.010 | 0.020 | 0.997 | 77-102 | 7.9 | 14.7 | 17 |
| 20 | Chlorpyriphos $C_9H_{11}Cl_3NO_3PS$ | 8.018 | 2921-88-2 | 349/97 | 197.0, 199.0, 201.0 | 0.002 | 0.010 | 0.998 | 88-101 | 7.5 | 15.6 | 21 |
| 21 | Triadimefon $C_{14}H_{16}ClN_3O_2$ | 8.072 | 43121-43-3 | 293/57 | 41.0, 208.0, 85.0 | 0.006 | 0.020 | 0.994 | 79-105 | 10.8 | 15.6 | 20 |
| 22 | Chlorfenvinfos α$C_{12}H_{14}Cl_3O_4P$ | 8.118 | 470-90-6 | 358/267 | 269.0, 29.0, 323.0 | 0.007 | 0.020 | 0.993 | 78-104 | 8.9 | 16.7 | 19 |
| 23 | Captan $C_9H_8Cl_3NO_2S$ | 8.205 | 133-06-2 | 299/79 | 77.0, 116.90, 149.0 | 0.004 | 0.020 | 0.997 | 83-102 | 8.5 | 16.5 | 21 |
| 24 | Chlorfenvinfos β$C_{12}H_{14}Cl_3O_4P$ | 8.221 | 470-90-6 | 358/267 | 269.0, 81.0, 323.0 | 0.005 | 0.020 | 0.995 | 79-103 | 3.4 | 12.6 | 17 |
| 25 | Quinalphos $C_{12}H_{15}N_2O_3PS$ | 8.249 | 13593-03-8 | 298/146 | 157.0, 156.0, 118.0 | 0.006 | 0.020 | 0.993 | 91-101 | 9.7 | 15.7 | 18 |
| 26 | Chlordane I $C_{10}H_6Cl_8$ | 8.302 | 57-74-9 | 406/372.8 | 374.8, 236.7, 271.7 | 0.004 | 0.010 | 0.995 | 77-102 | 9.8 | 13.8 | 20 |

**Table 1**. (*Continued*)

| S. No. | Name | RT | CAS NO. | Mol. weight, Target ion | SIM ions | LOD | LOQ | $r^2$ | % Recovery | Intra-day precision | Inter-day precision | ±UM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **GC-MSD- µECD –DFPD** | | | | | | | | | | | | |
| 27 | Jodfenphos $C_8H_8Cl_2IO_3PS$ | 8.314 | 18181-70-9 | 412/377 | 125.0, 379.0, 109.0 | 0.005 | 0.017 | 0.997 | 87-110 | 5.8 | 14.8 | 16 |
| 28 | O,pDDE $C_{14}H_8Cl_4$ | 8.390 | 3424-82-6 | 352/246 | 248.0, 318.0, 316.0 | 0.003 | 0.010 | 0.998 | 92-102 | 9.2 | 13.5 | 20 |
| 29 | Chlordane II $C_{10}H_6Cl_8$ | 8.415 | 57-74-9 | 406/374.8 | 372.9, 236.9, 271.7 | 0.004 | 0.010 | 0.993 | 77-98 | 9.8 | 12.8 | 18 |
| 30 | α-Endosulfan $C_9H_6Cl_6O_3S$ | 8.427 | 959-98-8 | 404/241 | 239.0, 195.0, 237.0 | 0.005 | 0.018 | 0.994 | 84-101 | 9.4 | 14.5 | 21 |
| 31 | Butachlor $C_{17}H_{26}ClNO_2$ | 8.578 | 23184-66-9 | 311/176 | 57.0, 160.10, 188.0 | 0.005 | 0.020 | 0.997 | 95-107 | 9.6 | 13.7 | 20 |
| 32 | P, P DDE $C_{14}H_8Cl_4$ | 8.809 | 72-55-9 | 316/246 | 317.9, 315.9, 248.0 | 0.003 | 0.010 | 0.996 | 76-107 | 9.1 | 15.1 | 19 |
| 33 | Dieldrin $C_{12}H_8Cl_6O$ | 8.932 | 60-57-1 | 378/79 | 81.0, 82.0, 77.0 | 0.007 | 0.020 | 0.995 | 78-103 | 9.7 | 16.8 | 19 |
| 34 | Profenofos $C_{11}H_{15}BrClO_3PS$ | 9.068 | 41198-08-7 | 372/337 | 339.0, 97.0, 139.0 | 0.010 | 0.020 | 0.998 | 93-105 | 4.6 | 14.2 | 18 |
| 35 | O, P DDD $C_{14}H_{10}Cl_4$ | 9.133 | 53-19-0 | 318/235 | 237.0, 165.1, 199.0 | 0.003 | 0.010 | 0.996 | 94-102 | 8.8 | 16.9 | 18 |
| 36 | Endrin $C_{12}H_8Cl_6O$ | 9.520 | 72-20-8 | 378/81 | 79.0, 263.0, 67.0 | 0.005 | 0.010 | 0.999 | 91-101 | 8.7 | 16.1 | 19 |
| 37 | β-Endosulphan $C_9H_6Cl_6O_3S$ | 9.768 | 33213-65-9 | 404/195 | 206.9, 236.8, 238.8 | 0.005 | 0.015 | 0.997 | 79-97 | 8.6 | 16.7 | 21 |
| 38 | P, P DDD $C_{14}H_{10}Cl_4$ | 9.826 | 72-54-8 | 318/235 | 237.0, 165.0, 199.0 | 0.003 | 0.010 | 0.999 | 76-99 | 8.9 | 16.9 | 22 |
| 39 | Ethion $C_9H_{22}O_4P_2S_4$ | 9.934 | 563-12-2 | 384/231 | 153.0, 96.90, 125.0 | 0.007 | 0.020 | 0.997 | 82-98 | 7.9 | 15.8 | 19 |
| 40 | Triazophos $C_{12}H_{16}N_3O_3PS$ | 10.311 | 789-02-6 | 313/161 | 77.0, 97.0, 162.0 | 0.004 | 0.020 | 0.995 | 79-95 | 9.4 | 16.9 | 20 |
| 41 | Endosulfan Sulphate $C_9H_6Cl_6O_4S$ | 10.772 | 1031-07-8 | 420/387 | 228.8, 169.8, 386.8 | 0.008 | 0.020 | 0.998 | 83-101 | 9.1 | 16.1 | 21 |
| 42 | Captafol $C_{10}H_9Cl_4NO_2S$ | 10.930 | 2425-06-1 | 347/79 | 80.0, 77.0, 78.0 | 0.010 | 0.020 | 0.995 | 75-102 | 9.8 | 16.8 | 19 |
| 43 | Chlorobenzilate $C_{16}H_{14}Cl_2O_3$ | 10.657 | 510-15-6 | 324/251 | 138.9, 110.9, 252.9 | 0.004 | 0.020 | 0.994 | 91-101 | 8.1 | 13.1 | 20 |
| 44 | Phosalone $C_{12}H_{15}ClNO_4PS_2$ | 12.222 | 2310-17-0 | 367/182 | 121.0, 97.0, 65.0 | 0.005 | 0.015 | 0.998 | 97-107 | 11.8 | 15.5 | 21 |
| 45 | Permethrin I $C_{21}H_{20}Cl_2O_3$ | 12.742 | 52645-53-1 | 390/183 | 162.9, 164.9, 77.0 | 0.005 | 0.015 | 0.995 | 91-112 | 10.1 | 16.9 | 22 |
| 46 | Permethrin II $C_{21}H_{20}Cl_2O_3$ | 12.973 | 52645-53-1 | 390/183 | 162.9, 164.9, 77.0 | 0.005 | 0.015 | 0.997 | 88-99 | 10.1 | 15.0 | 20 |
| 47 | Cypermethrin I $C_{22}H_{19}Cl_2NO_3$ | 13.891 | 52315-07-8 | 415/163 | 181.0, 164.9, 91.0 | 0.004 | 0.016 | 0.998 | 90-103 | 7.5 | 16.3 | 18 |
| 48 | Cypermethrin II $C_{22}H_{19}Cl_2NO_3$ | 14.028 | 52315-07-8 | 415/163 | 181.0, 164.9, 91.0 | 0.005 | 0.015 | 0.999 | 87-102 | 8.9 | 12.3 | 18 |
| 49 | Fenvalerate I $C_{25}H_{22}ClNO_3$ | 14.744 | 51630-58-1 | 419/125 | 167.0, 181.0, 151.9 | 0.006 | 0.017 | 0.994 | 77-104 | 11.7 | 14.8 | 19 |
| 50 | Esfenvalerate $C_{25}H_{22}ClNO_3$ | 14.961 | 66230-04-4 | 419/125 | 167.0, 181.0, 152.0 | 0.007 | 0.017 | 0.996 | 75-102 | 5.9 | 11.7 | 16 |

**Table 1**. (*Continued*).

| S. No. | LC-ESI-MSMS | RT | CAS NO. | Precursor ion | Product ion | LOD | LOQ | $r^2$ | % Recovery | Intraday precision | Inter-day precision | ±UM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 51 | Paraquat dichloride $C_{12}H_{14}Cl_2N_2$ | 9.740 | 1910-42-5 | 257.2 | 211, 175.1, 84.2 | 0.001 | 0.005 | 0.997 | 91-102 | 2.9 | 11.1 | 16 |
| 52 | Imidacloprid $C_9H_{10}ClN_5O_2$ | 9.712 | 13826-41-3 | 256 | 209, 175 | 0.001 | 0.005 | 0.998 | 97-107 | 1.8 | 12.8 | 19 |
| 53 | Diuron $C_9H_{10}Cl_2N_2O$ | 13.299 | 330-54-1 | 233 | 160, 72 | 0.004 | 0.015 | 0.991 | 73-91 | 3.1 | 14.0 | 22 |
| 54 | Isoproturon $C_{12}H_{18}N_2O$ | 13.143 | 34123-59-6 | 207.3 | 165.2, 133.8, 72.1 | 0.001 | 0.005 | 0.995 | 95-104 | 4.8 | 11.8 | 19 |
| 55 | Carbaryl $C_{12}H_{11}NO_2$ | 12.906 | 63-25-2 | 202 | 145, 127 | 0.001 | 0.005 | 0.996 | 86-106 | 5.1 | 12.1 | 20 |
| 56 | Carbendazim $C_9H_9N_3O_2$ | 6.740 | 10605-21-7 | 192.1 | 160, 105 | 0.003 | 0.011 | 0.998 | 89-98 | 4.8 | 10.1 | 18 |
| 57 | Benomyl $C_{14}H_{18}N_4O_3$ | 6.791 | 17804-35-2 | 192 | 160, 132 | 0.002 | 0.007 | 0.999 | 75-95 | 5.5 | 15.0 | 21 |
| 58 | Aldicarb $C_7H_{14}N_2O_2S$ | 11.252 | 116-06-3 | 116 | 89.1, 70 | 0.002 | 0.007 | 0.994 | 87-93 | 3.9 | 8.9 | 13 |
| 59 | 2,4-D $C_8H_6Cl_2O_3$ | 13.091 | 94-75-7 | 220 | 161, 163 | 0.001 | 0.005 | 0.998 | 81-94 | 2.7 | 11.7 | 17 |

[a] Compound Name, Retention time (min), CAS No, Molecular weight with Target and SIM ions, Limit of detection and quantification ((LOD & LOQ in mg/kg), coefficient of regression ($r^2$), Recoveries (%) (RSD %, n = 24 commodities), Repeatability as Intra-day and Inter-day precision expressed as % pooled RSD and overall uncertainties expressed as % (k=2) calculated at LOQ level.

This device provided a means of removing or changing the column without needing to cool and vent the mass spectrometer; gave protection against unwanted air entry while carrying out routine maintenance on columns and inlets; and offered a means for back flushing columns to remove high-boiling components, thus reducing both run times and cool-down times, as well as minimizing ghosting from run to run. Back flush is a means of discarding high-boiling compounds from a column after the peaks of interest have eluted. It saves analysis time and has the following additional benefits: longer column life (due to less high-temperature exposure), protection from air and water at high temperatures, and less chemical background and contamination of the MSD source. The advantage of using back flush in the column was demonstrated for two different matrices: mint and orange. Ten replicates were made for each extract, five with and five without back flushing. Both matrices showed the same results for the replicates run with back flush. However, for the replicates of both the matrices run without back flush, the baseline increased and retention times shifted ±10 s after three injections.

The used three-way splitter enhances productivity by splitting column effluent proportionally to multiple detectors: MSD, dual flame photometric detector (DFPD) and micro-electron capture detector (μECD). Therefore, two GC detector signals were acquired together with the MS data (both SIM and scan signals if desired) from one injection. This multi signal configuration provides full-scan data for library searching, SIM data for trace analysis, DFPD (phosphorus or sulfur mode) and μECD data for excellent selectivity and sensitivity from complex matrices (Supp. File, Figure S3). Here, an analyte would have similar retention times in all three detectors (for example in Supp. File, Figure S4 the screener software window for positive detection, identification and quantification of phosalone showed RT = 12.203 ± 0.02 min in spiked orange extract and same was identified by DRS and quantified by ChemStation).

Therefore, the GC data can be used in two ways: first, to confirm the presence of target analytes found by the MSD Deconvolution reporting software (DRS) (Table 2), and second, to highlight potential target compounds at low concentration to be further confirmed by ECD and DFPD.

Obtained chromatogram of fortified orange in trace ion mode of GCMS (Supp. File, Figure S4) was evaluated by DRS A.03.00 Deconvolution software. First the GCMS software i.e. MSD ChemStation E.02.00 performed a normal quantitative analysis for target pesticides using a target ion and three qualifier ions and the amount was reported for all calibrated compounds (available in Quantization database) that are detected. The DRS then sent the data file to AMDIS version 2.64 (Automated Mass Spectral Deconvolution and Identification software) provided by the National Institute of Standards & Technology with conventional NIST'05 MS library [27]. It deconvoluted the data, examined the intensity alterations of detected fragments, subtracted the matrix components from the spectra and the resulting purified spectrum was searched against mass spectral database i.e. the home amended RTL Pest library (RTL A.01.00), where a filter was set to fell the RTs in specified window. Therefore, the capillary GC analysis was in all cases performed under retention time locked (RTL) conditions, eluting the RTL calibrating solute parathion methyl at a constant retention time of 7.170 min. The presence of pesticides was then examined automatically via the RTL screener software in combination with the RTL-MS library for pesticides and endocrine disruptors, selecting four qualifier ions for positive identification. Because RTL is used to reproduce the RTL database retention times with high precision, this window is quite small (typically 10-20 s). Finally, the deconvoluted spectrum for the entire target found by AMDIS was searched against 147,000 compounds of NIST D.05.01 library for confirmation and it is retention time independent. The regular identification methods were comparatively more complex and time consuming. DRS eliminates many false positives and gives more confidence in compound identification by matching the deconvoluted data from two different libraries simultaneously [29,30]. The DRS report (Table 2) shows the screening and quantitation report of the sample in a single format, which takes less analysis time and gives more confidence in results than the built-in features of the data evaluation software. Quantitations for GC enable pesticide were done by both MSD and respective selective detector and thus the obtained results were found with standard deviation within ± 2 %.

### *3.3. Multi-residue screening and quantification of pesticides by LC-ESI-MS/MS*

The ESI is a very powerful and reliable LC-MS/MS source that has been introduced commercially. Depending on the source design, APCI works equally well or better as ESI for many pesticides but APCI heats the analytes more than ESI, which potentially leads to problems for thermo labile pesticides. Thus, ESI has greater analytical scope and has become the primary ionization technique in LC/MS. Due to the soft ionization nature of ESI, high background of LC mobile phases and relatively low separation efficiency of LC, tandem MS (and/or high resolution) is often required to determine

**Table 2.** MSD Deconvolution Report for spiked orange sample. The NIST library was searched for the components that were found in the AMDIS target library.

| R.T. | CAS # | Compound Name | Agilent ChemStation Amount (ppm) | AMDIS Match | AMDIS R.T. Diff sec. | NIST Reverse Match | NIST Hit Num. |
|---|---|---|---|---|---|---|---|
| 2.7944 | 1113026 | Omethoate | - | 72 | <20 | 79 | 2 |
| 2.8103 | 100185 | Benzene, 1,4-bis(1-methylethyl)- | - | - | <20 | 92 | 1 |
| 3.0534 | 623916 | 2-Butenedioic acid (E)-, diethyl ester | - | - | <20 | 96 | 1 |
| 3.5892 | 7786-34-7 | Dichlorvos | 0.077 | 95 | <20 | 92 | 2 |
| 3.6479 | 90120 | Naphthalene, 1-methyl- | - | - | <20 | 82 | 1 |
| 3.7969 | 717748 | Benzene, 1,3,5-tris(1-methylethyl)- | - | - | <20 | 79 | 1 |
| 3.9935 | 2040053 | 2,6-Dichloroacetophenone | - | - | <20 | 80 | 1 |
| 4.3143 | 2032657 | Methiocarb | - | 86 | <20 | - | - |
| 4.5399 | 28839498 | 1H-Isoindole-1,3(2H)-dione, 4,5,6,7-tetrahydro-2-methyl | - | - | <20 | 85 | 1 |
| 4.6620 | 0000 | l-Alanine, N-(2,6-difluorobenzoyl)-, hexyl ester | - | - | <20 | 83 | 1 |
| 4.7027 | 626437 | 3,5-Dichloroaniline | - | 98 | <20 | - | - |
| 4.7027 | 95761 | Benzenamine, 3,4-dichloro- | - | - | <20 | 95 | 1 |
| 5.0441 | 27813214 | Tetrahydrophthalimide, cis-1,2,3,6- | - | 95 | <20 | - | - |
| 5.0451 | 6923224 | Monocrotophos | 0.081 | 97 | <20 | 94 | 1 |
| 5.0732 | 6108107 | BHC epsilon isomer | 0.098 | 86 | <20 | - | - |
| 5.0732 | 28903244 | Cyclohexene, pentachloro- | - | - | <20 | 92 | 1 |
| 5.2185 | 90153 | 1-naphthalenol | 0.079 | 98 | <20 | 93 | 1 |
| 5.5107 | 4185824 | Phosphoric acid, dimethyl 1-methylethenyl ester | - | - | <20 | 72 | 1 |
| 5.7004 | 298022 | Phorate | 0.103 | 90 | <20 | 89 | 2 |
| 5.7152 | 39515510 | Benzaldehyde, 3-phenoxy- | - | - | <20 | 92 | 1 |
| 5.8029 | 319846 | BHC alpha isomer | 0.080 | 99 | <20 | 93 | 2 |
| 5.8294 | 640153 | Thiometon | 0.083 | 88 | <20 | 92 | 2 |
| 5.8801 | 100027 | 4-Nitrophenol | 0.091 | 88 | <20 | 86 | 2 |
| 6.1380 | 20925853 | Benzonitrile, pentachloro- | - | - | <20 | 81 | 1 |
| 6.1639 | 319857 | BHC beta isomer | 0.093 | 99 | <20 | 82 | 1 |
| 6.3341 | 60515 | Dimethoate | 0.088 | 95 | <20 | 91 | 2 |
| 6.6189 | 84695 | Diisobutyl phthalate | - | 90 | <20 | 90 | 1 |
| 6.816 | 58899 | Lindane | 0.086 | 88 | <20 | 76 | 1 |
| 6.1639 | 319857 | BHC delta isomer | 0.096 | 90 | <20 | 82 | 1 |
| 6.8197 | 1897456 | Chlorothalonil | 0.101 | 96 | <20 | 90 | 2 |
| 6.9196 | 34256821 | Acetochlor | 0.084 | 72 | <20 | 71 | 1 |
| 6.9344 | 95250 | Chlorzoxazone | - | - | <20 | 73 | 1 |
| 6.952 | 319868 | BHC delta isomer | 0.103 | 83 | <20 | 94 | 1 |
| 7.006 | 1897456 | Chlorthalonil | 0.099 | 81 | <20 | 91 | 1 |
| 7.0270 | 2540821 | Formothion | 0.105 | 90 | <20 | 81 | 2 |
| 7.170 | 298000 | Methyl parathion | 0.077 | 91 | <20 | 92 | 2 |
| 7.227 | 76448 | Heptachlor | 0.087 | 98 | <20 | 91 | 1 |
| 7.3033 | 84742 | Di-n-butylphthalate | - | 84 | <20 | 84 | 1 |
| 7.5300 | 122145 | Fenitrothion | 0.104 | 89 | <20 | 85 | 1 |
| 7.5934 | 309002 | Aldrin | 0.086 | 92 | <20 | 88 | 2 |
| 7.6130 | 121755 | Malathion | 0.092 | 93 | <20 | 92 | 2 |
| 7.6606 | 85290 | 2,4'-Dichlorobenzophenone (2,4'-Dicofol decom.product) | - | 85 | <20 | 90 | 2 |
| 7.717 | 55389 | Fenthion | 0.081 | 92 | <20 | 87 | 1 |
| 7.884 | 56382 | Parathion | 0.079 | 94 | <20 | 90 | 1 |
| 8.018 | 2921882 | Chloropyriphos | 0.100 | 91 | <20 | 87 | 1 |
| 8.072 | 43121433 | Triadimefon | 0.107 | 88 | <20 | 79 | 1 |
| 8.118 | 470906 | Chlorfenvinfos alpha | 0.096 | 89 | <20 | 77 | 1 |
| 8.209 | 133062 | Captan | 0.092 | 82 | <20 | 73 | 1 |
| 8.221 | 470906 | Chlorfenvinfos beta | 0.082 | 91 | <20 | 76 | 1 |
| 8.249 | 13593038 | Quinalphos | 0.087 | 88 | <20 | 71 | 1 |
| 8.2836 | 3424826 | o,p'-DDE | 0.093 | 97 | <20 | 92 | 2 |
| 8.302 | 57749 | Chlordane-I | 0.105 | 77 | <20 | 78 | 1 |
| 8.314 | 18181709 | Jodfenphos | 0.099 | 82 | <20 | 76 | 1 |
| 8.415 | 57749 | Chlordane - II | 0.089 | 83 | <20 | 74 | - |
| 8.4210 | 959988 | Endosulfan (alpha isomer) | 0.091 | 98 | <20 | 72 | 1 |
| 8.5853 | 5103742 | trans-Chlordane | 0.081 | 82 | <20 | 78 | 2 |
| 8.6810 | 39765805 | Nonachlor, trans- | 0.079 | 83 | <20 | 83 | 2 |
| 8.7486 | 15972608 | Alachlor | 0.102 | 73 | <20 | - | - |
| 8.7486 | 23184669 | Butachlor | 0.106 | 97 | <20 | 91 | 1 |
| 8.8031 | 72559 | p,p'-DDE | 0.098 | 92 | <20 | 89 | 2 |
| 8.9288 | 60571 | Dieldrin | 0.104 | 96 | <20 | 92 | 2 |
| 9.0535 | 66870891 | p-Tolylpentamethyl-disiloxane | - | - | <20 | 77 | 1 |
| 9.068 | 41198087 | Profenofos | 0.092 | 82 | <20 | 76 | 1 |
| 9.520 | 72208 | Endrin | 0.102 | 87 | <20 | 79 | 1 |
| 9.6428 | 510156 | Chlorobenzilate | 0.104 | 96 | <20 | 93 | 2 |
| 9.7786 | 33213659 | Endosulfan (beta isomer) | 0.082 | 78 | <20 | 76 | 1 |
| 9.8177 | 53190 | o,p'-DDD | 0.088 | 98 | <20 | 94 | 2 |
| 9.826 | 72548 | P, P DDD | 0.098 | 88 | <20 | 77 | 1 |
| 9.9324 | 563122 | Ethion | 0.103 | 95 | <20 | 89 | 2 |
| 10.311 | 789026 | Triazophos | 0.093 | 97 | <20 | 88 | 1 |
| 10.7732 | 1031078 | Endosulfan sulfate | 0.092 | 95 | <20 | 85 | 1 |
| 10.930 | 2425061 | Captafol | 0.085 | 97 | <20 | 76 | 1 |
| 11.6235 | 117817 | Bis(2-ethylhexyl)phthalate | - | 73 | <20 | - | - |
| 11.6235 | 4376209 | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester | - | - | <20 | 78 | 1 |
| 12.2037 | 2310170 | Phosalone | 0.098 | 96 | <20 | 90 | 1 |
| 12.742 | 52645531 | Permethrin - I | 0.087 | 93 | <20 | 84 | 1 |
| 12.973 | 52645531 | Permathrin - II | 0.091 | 96 | <20 | 97 | 1 |
| 13.891 | 52315078 | Cypermethrin I | 0.105 | 73 | <20 | 76 | 1 |
| 14.1648 | 52315078 | Cypermethrin II | 0.093 | 81 | <20 | 84 | 1 |
| 14.7414 | 66230044 | Esfenvalerate | 0.080 | 89 | <20 | 91 | 1 |
| 14.9620 | 51630581 | Fenvalerate I | 0.106 | 87 | <20 | 76 | 1 |

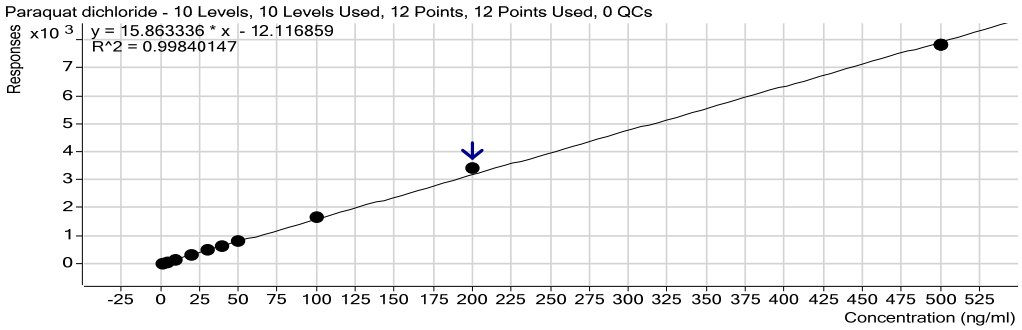


**Figure 1.** Linearity graph of paraquat dichloride for 10 levels.

pesticide residues in complex extracts. Here the optimization of LC-MS/MS (ESI) conditions was carried out in two parts. First was to optimize the fragmentor voltage for each of the 9 compounds in order to produce the greatest signal for the precursor ion. Each compound was analyzed separately using an automated procedure to check the fragmentor at each voltage. The data were then selected for optimal fragmentor (80-90 V) signal and each compound was injected in a programmed run at a concentration of 1 µg/mL to determine the collision energies for both the quantifying and qualifying ions. Various collision energies (5, 10, 15, 20, 25 and 30 V) were applied to the compounds under study. The energies were optimized for each of the ions and the voltages that give the best sensitivity were selected. The MRM transition used for each is shown in Table 1 for all the 9 studied compounds. Quantization was based on external standardization by employing calibration curves in the range of 1 - 500 ng/mL based on the peak area calculated from selected ion chromatograms (one precursor with two qualifier ions) of the corresponding $[M-H]^+$ ion. Results were expressed as mg/kg.

### *3.4. Method performance*

#### *3.4.1. Limits of detection, limits of quantification and linearity*

The LOD is the lowest concentration of the analytes in a sample, which can be detected but not necessarily quantified. The LOQ is the lowest concentration of the analytes in a sample, which can be quantified with an acceptable degree of accuracy and precision. The LODs and LOQs have been established by analyzing 10 replicates of each sample blanks. The LOD has been calculated as a signal to noise ratios of 3 and verified as three times the standard deviation (SD) of the obtained noise (LOD = 3×SD). The LOQ was defined as the analytes concentration resulting in S/N of 10 and verified by the afore-mentioned procedure applied for LOD. LOQ equals to "mean + 10 × SD". The value of "mean" is the average of concentration levels determined from the blank signals in the 10 independent replicates by the same analysis procedures [31]. As shown in Table 1, the calculated limits of quantification for the majority of the compounds in different groups are below ≤0.020 mg/kg. Finally, the reported LOQ is taken as the concentration with the acceptable precision and accuracy of the measurement. At LOQ, the samples were analyzed for their repeatability, robustness and recovery estimation and were accepted with less than 17 % relative standard deviation (RSD) and with 75-107 % of recovery. The reported LOQ (Table 1) for organophosphorous pesticides are higher than other compounds but all are lower than the Maximum Residue Levels (MRL) stipulated in the Prevention Food Adulteration Act, (PFA, 2009) for fruits and vegetables.

The linearity of the method was obtained by least-squares linear regression analysis of the peak area versus analytes concentration, using seven concentration levels (0.010, 0.025, 0.050, 0.100, 0.200, 0.250 and 0.500 mg/kg) for GCMS along with ECD (for Organo chlorine compounds) and DFPD (for organophosphorous compounds) and ten concentrations (1, 5, 10, 20, 30, 40, 50, 100, 200, and 500 ng/mL or ng/g) for LC-MS/MS analyzed compounds in duplicates (Figure 1).

The correlation coefficients ($r^2$) are shown in (Table 1), with high values of ($r^2 > 0.991$) and excellent linearity being obtained for the range studied.

#### *3.4.2. Repeatability, intermediate precision and robustness*

The repeatability of an analytical method refers to the use of the procedure within a laboratory over a short period of time, carried out by the same analyst with the same equipment. According to the International Conference on Harmonization (ICH), it is recommended that repeatability be assessed using a minimum of nine determinations covering the specified range (i.e., three concentrations and three replicates for each concentration) or a minimum of six determinations of 100 % of the test concentration [31].

The intra-day accuracy and repeatability was assessed, at three concentration levels with six replicates for each concentration on the same day. Table 1 shows the mean repeatability of the method for the investigated compounds in the spiked samples (24 commodities). The results show that the RSD of intra-day precision ranged between 2.8 % and 9.8 %.

The intermediate precision in this study is based on the mean repeatability values of a set of spiked samples at three concentration levels and analyzed daily for a period of 3 days. The RSD values of inter-day precision ranged from 5.9 % to 17.0 % for three groups (24 commodities) indicating that the proposed GC-MSD-µECD-DFPD and LC-MS/MS method produces acceptable intermediate precision and accuracy.

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. It is evaluated in this method by varying method parameters such as increasing the extraction up to 10-15 minutes and by delaying the analysis time for one-day after completing the extraction procedure. The results of inter day precision shows good robustness of the method with a mean value as % RSD of less than 17 %. The above two parameters were as evaluated as the stability of analytical solution and the extraction time are two typical variations [31].

#### *3.4.3. Specificity and recovery*

Specificity is the ability to assess unequivocally the analytes in the presence of impurities, degradants, matrices, etc. In this analytical method, specificity is proved by comparing the chromatograms of a set of blank and spiked matrix solutions, which revealed that the require analytes eluted > 75 % with relative standard deviate < 17 % (Figure 2 and 3).

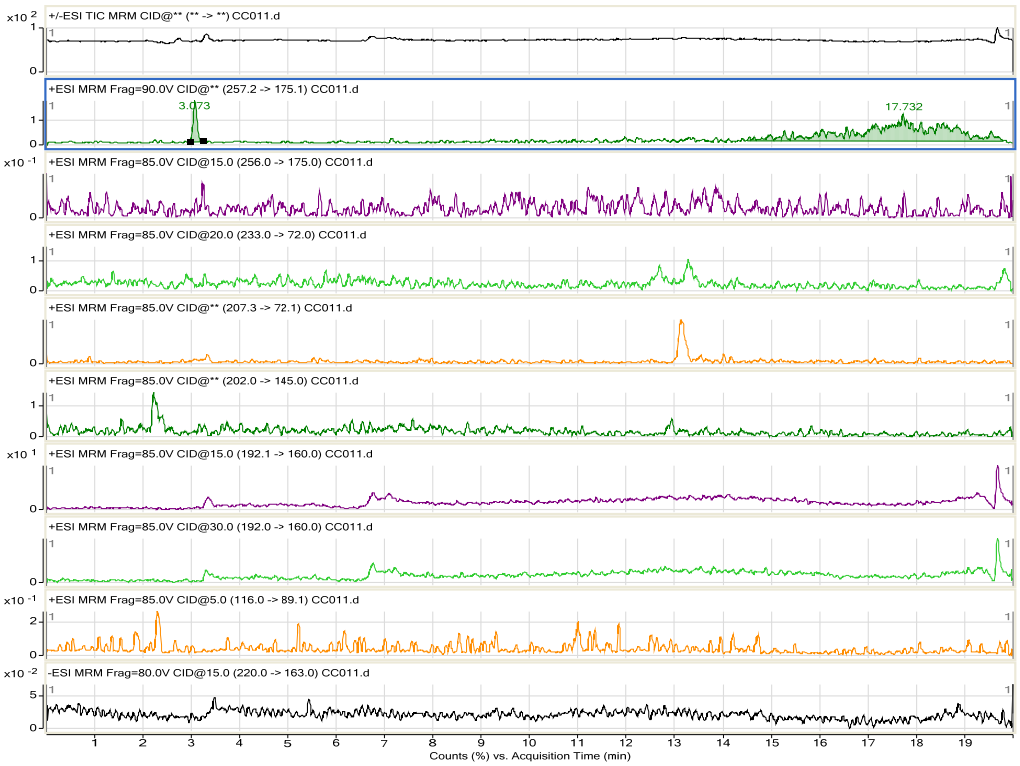


**Figure 2**. Chromatograms of a blank matrix analyzed by LC-MS/MS for nine pesticides.

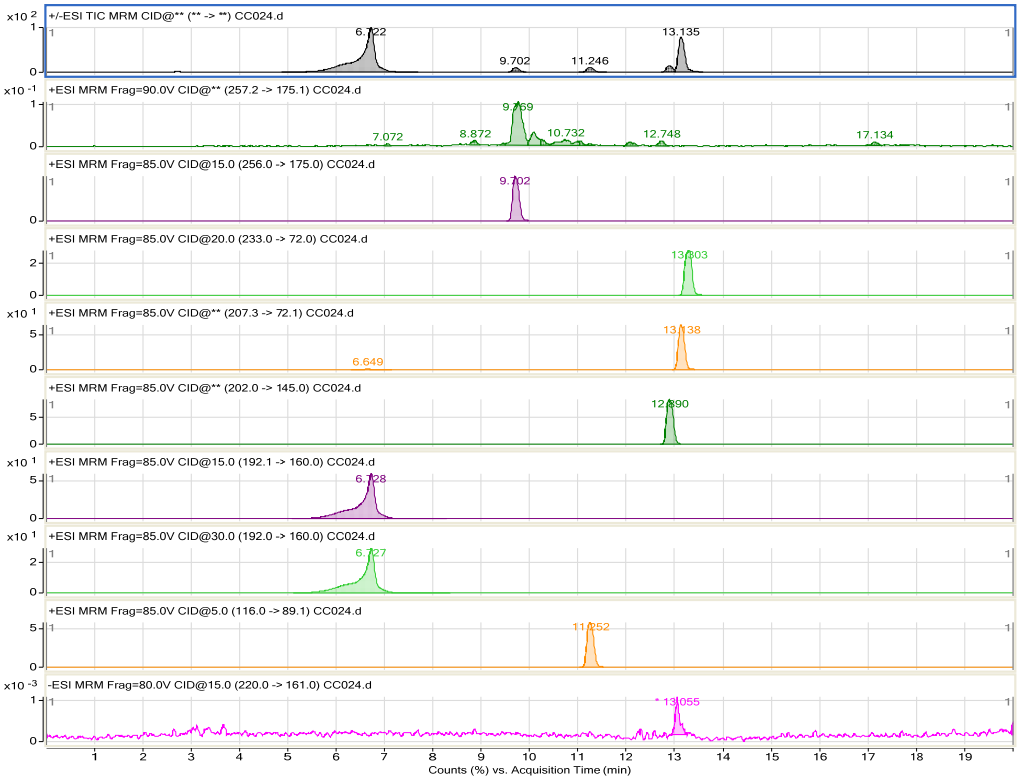


**Figure 3**. Chromatograms of a spiked matrix analyzed by LC-MS/MS for nine pesticides.

**Table 3**. Pesticide levels detected in selected fruits and vegetables. [Results: mg/kg ± SD].

| Name | Beans n =2 | Eggplant n = 18 | Okra n =5 | Radish n =2 | Cauliflower n =11 | Cabbage n =10 | Capsicum n =4 | Grapes n =8 | Tomato n =11 | Apple n =2 |
|---|---|---|---|---|---|---|---|---|---|---|
| Formothion | ND[a] | ND | ND | ND | ND | 0.05 | ND | 0.04±0.04 | ND | ND |
| Methyl Parathion | ND | ND | ND | ND | ND | ND | 0.08±0.03 | ND | 0.12±0.05 | ND |
| Fenitrothion | ND | 0.33±0.17 | 0.41±0.16 | ND | 0.08±0.05 | ND | 0.16±0.17 | ND | 0.33±0.17 | ND |
| Malathion | ND | ND | ND | 0.22±0.08 | 0.22±0.12 | ND | ND | ND | 0.19±0.12 | ND |
| Fenthion | ND | 0.06±0.01 | ND | ND | ND | ND | ND | ND | ND | ND |
| Parathion | ND | ND | ND | ND | 0.11±0.04 | ND | ND | ND | ND | ND |
| Chlorpyriphos | ND | 0.13±0.27 | 0.33±0.17 | ND | 0.23±0.19 | 0.41±0.1 | 0.33±0.17 | 0.33±0.17 | 0.33±0.17 | 0.13±0.10 |
| Captan | ND | ND | 0.04±0.01 | ND | ND | ND | ND | ND | ND | ND |
| Endosulfan(total) | ND | ND | ND | ND | ND | ND | ND | 0.13±0.07 | ND | ND |
| P, P DDE | ND | ND | ND | ND | ND | ND | ND | 0.019±0.01 | ND | ND |
| P, P DDD | ND | ND | ND | ND | ND | 0.02±0.01 | ND | ND | ND | ND |
| Ethion | ND | ND | ND | ND | 0.12±0.01 | 0.07±0.01 | ND | ND | 0.08±0.01 | ND |
| Phosalone | ND | ND | 0.05±0.02 | ND | ND | ND | ND | 0.03±0.01 | ND | ND |
| Cypermathrin (total) | ND | 0.23±0.11 | ND | ND | ND | 0.14±0.04 | ND | ND | ND | 0.11±0.06 |
| Fenvalerate (total) | ND | 0.07±0.17 | 0.33±0.17 | ND | ND | ND | ND | ND | ND | ND |
| Paraquat dichloride | 0.03±0.01 | ND | ND | ND | ND | ND | ND | 0.05±0.02 | ND | ND |
| Carbaryl | ND | ND | 0.05±0.02 | ND | 0.11±0.03 | 0.13±0.04 | ND | ND | ND | ND |
| Carbendazim | ND | ND | ND | ND | 0.08±0.01 | ND | ND | 0.12±0.01 | ND | ND |
| Benomyl | 0.21±0.1 | ND | ND | ND | ND | ND | ND | ND | ND | ND |

[a]ND: Not detected.

By using the stated method, acceptable relative recoveries were obtained, ranging between 75 and 107 % (intermediate accuracies ranging from 74.3 to 109.2% with the RSD values from 2.8 to 9.8 % and inter-day accuracies from 73.6 to 110.2 % with the RSD values from 5.9 to 17.0 %) and for the analysis of pesticide residues at the ppb or ppm levels, accuracy or recovery of 70 to 120 % is considered as acceptable [32]. Hence, the results obtained above can be considered to be acceptable for the concentration levels being investigated. The mean recovery data and its % RSD values obtained in the analysis of 24 fortified fruit and vegetable samples are as listed in Table 1.

### *3.4.4. Overall uncertainties*

Due to difficulty in calculating the individual uncertainty contributions following a "bottom up" procedure, as proposed by the ISO guide [28] the different contributions were grouped as recommended by the EURACHEM/CITAC guide. The contributions in the MAE-(d-SPE)-RTL-GC-MS method can be grouped in three terms, permitting the calculation of the overall uncertainty according to the following equation:

$$Ur = (r \times k)\ sqrt((u\ (CRM))^2 + (u\ (Rep))^2 + (u\ (Bias))^2) \quad (1)$$

The first term ($u$CRM) corresponds to the relative uncertainty from the certified reference material used for calibration and the subsequent uncertainties introduced by the balance, volumetric material, etc. during weighing and diluting to the final concentration. The second term ($u$ (Rep)) corresponds to the relative uncertainty of contribution due to the precision of the method, also called repeatability uncertainty, which gives a value for the standard uncertainty due to run-to-run variation, day-to-day variation, analyst-to-analyst variation and commodity-to-commodity variation of the overall analytical process. ($u$ (Bias)) is the relative uncertainty due to *bias* i.e. corresponds to the tolerance that each laboratory establishes for their internal quality controls of the analytical procedure, investigated during the in house validation study using spiked samples (homogenized sample were split and spiked). Finally, $k$ and r are the coverage factor and reported results respectively to expand the uncertainty to the desirable level of confidence with desirable units of measurement. The second and third terms are generally the most important contributions to the overall uncertainties. In the present work, the overall uncertainties were calculated at 0.05 mg/kg level. The $u$CRM was calculated by taking into account all the dilution steps and the uncertainties from the CRM and all the volumetric material and balances used to prepare the calibration standards included the tolerance that our laboratory accepts as a maximum for the verification of the daily calibration curve. Those were calculated from the $n$= 20 results (each matrix) from the experiment performed under repeatable and reproducible conditions. The third term was calculated considering mean recovery of samples with recovery from 75 to 107 % with a relative standard deviation of less than 17 % (tolerance that the laboratory accepts as a maximum for the verification of the daily analysis). Finally, a coverage factor $k$ = 2 was used for a confidence interval of 95 % ($n$ = 9×20). As shown in Table 1, the uncertainties were calculated for 0.05 mg/kg.

### *3.5. Screening real market samples*

The developed GC-MSD-ECD-DFPD and LC-ESI-MS/MS method has been applied to the analysis of 403 vegetables obtained from a local market. Table 3 shows the pesticide levels detected in the selected vegetable samples. Out of these 403 samples only 73 samples were found positive for pesticides and the obtained residue level for 41 were found to be lower than the limits of PFA, 2009 (Table 3).

To ensure the validity of the results when the proposed method is applied for routine analysis; quantification of each sample was made with the corresponding matrix-matched calibration plot, depending on the specific commodity category. All samples shown in this table were analyzed by DRS; the match values obtained were higher than 60 %, and retention time differences between the pesticide database and observed values were < 10 s. In addition, all positive results given by AMDIS were confirmed as being positive by the NIST library. The internal quality control criteria were also applied in order to check if the system is under control: a blank extract was carried out daily to eliminate any false positive via contamination in the extraction process, instrument or

reagents used. A blank extract spiked at the intermediate concentration level was run prior to the analysis of the real sample in order to assess the extraction efficiency.

## 4. Conclusion

Indeed, the concurrent use of LC/MS-MS and (LVI)/GC-MS/μECD/DFPD for nearly any pesticide constitutes the state-of-the-art approach to multiclass, multi residues analysis of pesticides in a variety of matrices. The QuEChERS method is an effective and efficient sample preparation procedure that produces sample extracts suitable for both of these powerful analytical tools. DRS solves the purpose of quick screening and quantification of multi residues in full scan and selected ion chromatograms of such complex matrices in a much shorter data analysis time and also helps chemists in decision making process. This method also provides accurate results for a variety of pesticides present in the food matrices as it obtains confirmation from two to three different detectors for GC and from triple quad for LC. It is demanding and sufficient for routine analytical purposes, which was earlier difficult with the built-in features of the data evaluation software. The validation results for the developed methods are comparable for various fruit and vegetable matrices. The excellent LOD and LOQ with the use of only 10 g sample weight, the good linearity, robustness, accuracy and reproducibility together with automation and simplicity than conventional method makes it a key tool for the routine quality control of above pesticides in fruits and vegetables as per the PFA, 2009 and Codex.

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### *Supplementary material*

Supporting information for this article is available on the WWW under http://www.eurjchem.com.

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