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ORIGINAL ARTICLE

Optimization and Validation of 51 Pesticide Residues in Fruits and Vegetables Using Ultra Performance Liquid Chromatography Tandem Mass Spectrometry

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ABSTRACT

A multiresidue method for the determination of 51 pesticide residues in commonly consumed fruits and vegetables including Apple, Grapes, Pepper, and Tomato, was optimized and validated. Samples were extracted by a quick, easy, cheap, effective, rugged and safe (QuEChERS) method then quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS). The parameters of the triple quadrupole mass spectrometer were optimized by selecting the proper CE parameters for the precursor and two products ions for quantification of each compound. The HPLC conditions were optimized using a mobile phase consists of 10 mmol L^{-1} ammonium formate at pH 4. Samples were extracted by shaking with acetonitrile. Phase separation was induced by shaking with buffer–salt mixture consisting of magnesium sulfate, sodium chloride, disodium hydrogen citrate sesquihydrate, and trisodium citrate dehydrate. The sample was centrifuged and an aliquot of the clear solution was injected into the LC-MS/MS system. Matrix matched standards were used to compensate for the matrix effect. Quantization and confirmation were attained by using atmospheric pressure electrospry positive ionization LC-MS/MS in multiple reactions monitoring (MRM) mode. The recoveries at three different concentration levels of 10, 50 and 100 µg kg^{-1} ranged from 70 to120% for most pesticides.

Keywords: *Pesticide residues, liquid chromatography, tandem mass spectrometry, fruits, vegetables*

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INTRODUCTION

Pesticides are a numerous and diverse group of chemical compounds that have being used for protecting the crops from diseases, insects and weeds, and contributing to produce the highly qualified agricultural food stuffs. But there is a possibility of threat to the public due to the effects of pesticide residues in the foodstuffs. [1]

Food-borne exposure to agricultural and environmental chemicals is of great public concern. Owing to the development of sensitive analytical methods of detection, trace amounts of potentially harmful chemicals can be detected in many foods. [2] The use of multiclass and multi-residue methods (MRMs) is the most efficient approach to pesticide residue analysis in terms of analysis costs and turnaround time. Many MRMs have been developed for the determination of pesticide residues in fruits and vegetables. The first notable MRM was the Mills method developed in the1960s. At that time, non-polar organochlorins insecticides (OCs) were the main focus for analysis. With the Mills method, OCs and other non-polar pesticides were extracted from non-fatty foods with acetonitrile (MeCN), which was then diluted with water, and the pesticides were partitioned into a non-polar solvent (petroleum ether). As a consequence, relatively polar pesticides, such ascertain organophosphorus insecticides (OPs), were partially lost during this step.[3] In the 1970s, new methods were developed to extend them analytical polarity range to cover OCs, OPs and organonitrogen pesticides (ONs) in a single procedure.

These methods include acetone extraction and liquid-liquid partitioning with dichloromethane.[4&5] Environmental and health concerns related to the use of chlorinated solvents have led to the development of many new methods in which such solvents were avoided. Specht *et al* .[6] used a mixture of cyclo-hexane–ethyl acetate (1 ± 1) instead of dichloromethane to induce partitioning. Casanova [7] used solid-phase extraction (SPE) to extract pesticides from diluted acetone extracts, thus completely avoiding liquid–liquid partitioning. Jansson *et al* .[8] used extraction with ethyl acetate in presence of sodium sulfate followed LC-MS/MS determination. The dramatic pace of innovation in instrumental analysis techniques during the past decade assisted in using simple preparation procedures, with the most significant impact in this respect being attributed to the LC/MS (/MS) technology, which opened the door for an easy and reliable analysis of numerous traditionally "difficult" pesticides. Anastassiades *et al* . [9] Recently introduced the so-called quick, easy, cheap, effective, rugged, and safe (QuEChERS) method of pesticide residue analysis. In a follow-up study, Lehotay *et al* . [10] demonstrated its effectiveness for >200 pesticides in lettuce, orange and several other matrices using gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) for analysis. Multiresidue methods and require specialized techniques for more accurate analysis (Angioni *et al* .[11] Di Muccio *et al* [12] and Gilvydis and Walters [13]. Lehotay *et al* . [14] used buffer mixture (sodium acetate in presence of acetic acid) at extraction step to overcome the degradation of problematic pesticides.

The use of acetic acid in buffer solution could decrease the efficiency of the clean up step using PSA; Anastassiades *et al* . [15] used disodium citrate and trisodium citrates buffering mixture. In the present study, we used the simple, rapid, and reliable multiresidue method for determination of different pesticides groups such as organophosphorus and pyrethroids in fresh samples (Grape, Apple, Tomato and pepper) using acetonitrile extraction before LC-MS/MS determination. Optimization and validation of the method have been carried out using the criteria of Codex Alementarious for validation; including identification of recoveries, limit of quantification, precision, accuracy and the uncertainty.

MATERIALS AND METHODS

Apparatus

PFTE or polyethylene 50 ml with screw cap tubes. Centrifuge (Thermo up to 4000 rcf). LC–MS/MS was performed with an Waters Aquity instrument as UPLC coupled to Xevo TQ-S MS/MS from Waters company with electrospray ionization (ESI) interface.

Chemical and reagents

Acetonitrile (HPLC, assay >99%), methanol HPLC grade (assay >99.9%), and formic acid (assay 98-100%), were received from Fluka (Switzerland). Ammonia solution, 33% was obtained from BDH(UK). Sodium chloride, 99%, Disodium hydrogencitratesesquihydrate,Trisodium citrate dehydrate, Sodium chloride and anhydrous magnesium sulphate (Bond ElutQuEChERS Extraction kit (Part No.: 5982-5650). De-ionized water produced by Milli-Q unit (Millipore).Buffer-salt-mixture used for second extraction and partitioning ready made by Agilent Technology as 4±0.2 g of anhydrous magnesium sulphate, 1±0.05 g of sodium chloride, 1±0.05 g of trisodium citrate dehydrate and 0.5±0.03 g of disodium hydrogencitratesesquihydrate into 25 ml glass tube. The LC mobile phase was prepared by mixing of 10 mmol L^{-1} ammonium formate solution in methanol-water with ratio of (0.2:9.8) at pH 4±0.1. Pesticide reference standards (purity >95%) were purchased from Dr. Ehrenstorfer (Augsburg,Germany).

A stock solution of 1000 µg mL^{-1} reference standard solutions of all the analyzed pesticides was prepared in methanol. Stock solution was kept at -18±2^{o}C.

Intermediate solution of individual standards of 1 µg mL^{-1} of pesticides was prepared by diluting stock solution in methanol. Calibration mixtures of concentration levels 0.005, 0.01, 0.05, 0.1 and 0.5 µg mL^{-1} were prepared in methanol

Extraction procedur

A 10 g (W) sample was weighed in 50 ml PFTE tube, and 10 ml of acetonitrile was added and shaken vigorously for one minute. Buffer-salt-mixture was added and shaken immediately for one minute. The sample was centrifuged at 4000 rpm for 5 minutes. Of the clear solution 4 mL was filtrated by Agilent PVDF (Part No.: 5982-5650) 0.45 um of the sample 2 µL was injected into

LC-MS/MS operating conditions

Separation was performed on a C18 column, Aquity UPLC BEH - C18 2.1 mm x 10 mm, 1.7 µm particle size. The injection volume was 2 µL. A gradient elution program was at a flow rate of 0.45 ml min^{-1}, in which one reservoir contained 10 mmol L^{-1} ammonium formate solution in methanol-water (0.2:9.8) and the other contained methanol.

The ESI source was used in the positive mode, and N2 nebulizer, cone, and other gas settings were optimized according to recommendations made by the manufacturer; source temperature was 200^{0}C, ion

spray potential 3300 V, Cone was 30 V, desolvation gas adjusted to 800 L/Hr and nebulizer at 7 Bar, collision energy were optimized using intellistart part individual pesticide solutions into the MS instrument to allow optimization of the MS/MS conditions, which are shown in **Table 1**. The Multiple Reactions Monitoring mode (MRM) was used in which one MRM was used for quantitation and other was used for confirmation.

RESULTS AND DISCUSSION

The data presented in **Table 2** revealed the program used for operating the ultra performance system (UPLC). The running time was 10 min with flow rate of 0.45 ml min^{-1}. the ratio of Aqua solution to organic changed with time as shown in the table. The retention times, quantifiers and the confirmers MRM's of tested pesticides were illustrated in **Table 1**, however, two MRM were selected for each.

The method recoveries for 51 pesticides were tested by performing 6 replicates of spiked (grape, apple, green pepper and tomato) samples at different concentration levels of 10, 50 and 100 µg kg^{-1}. The average recoveries and relative standard deviation on each level were calculated and listed in Table 3.

Optimization condition and recoveries

The optimization conditions of UPLC as showed in **Table 1**. Different mobile phase solutions for HPLC at different concentrations were tested. The optimum operating conditions were achieved using a mobile phase solution of 10 mmol L^{-1} ammonium formate at pH 4 which had led to good separation and obtaining of sharp peaks, this findings were in agreement with Jansson *et al* .[8] who reported that the best signal response could be obtained at a pH range of 4.0 to 4.2. The buffer strength of 10 mmol L^{-1} was selected as a compromise condition for the detection of 51 pesticides residues. **Figure 1** shows the obtained spectrum of LC-MS/MS for spiked Apple with selected pesticides at a concentration of 50 µg L^{-1}. Also, the use of buffer helps to have more stable pesticides separation and the adjusted pH prevent pesticides from degradation according to pesticides stability in pH referenced by the British Crop Protection Council [16]. Tandem mass optimization was done as showed in **Table 1**. The quantifier and confirmer ion was selected as a conformation tool of the obtained results in case of two ions appeared in same retention time and if ion ratio was less than 30%.

Table (1) Liquid phase gradient using ultra performance system

Time (min)	(A) % Aqua	(B) % Organic	Flow Rate (ml/min)
initial	**90**	**10**	**0.45**
0.25	90	10	0.45
7.75	5	95	0.45
8.5	5	95	0.45
8.51	90	10	0.45
10	90	10	0.45

The method recovery (R) for 51 pesticides was tested by performing 6 replicate measurements of spiked fruit extracts of grape, apple, green pepper and tomato samples at concentration levels of 10 and 50 µg kg^{-1}. The recovery percentage R(%) and relative standard deviation (RSD%) at the concentration level of 10 µg kg^{-1} are compiled in **Table** 3. Also, a comparison diagram for the recovery (%) obtained at 50 µg kg^{-1} spiked pesticides is presented in **Fig. 2**. The selected compounds covered wide range of pesticides polarity and physic-chemical properties, starting from the highest polar pesticides (Methamidophos) to lowest polar pesticide (Tefluthrin) to confirm the method performance with the obtained small run time of 10 min. The lowest recoveries were observed for the 3 pesticides Sulfotep, Bifenthrin and Paraoxon-Methyl with R(%) values less than 70%. Generally, all measured samples showed low sensitivity using MRM mode for those 3 pesticides. This was clearly observed with Sulfotep in grape and apple where only the spiked level of 100 µg kg^{-1} could be detected. But for pepper, the high suppression effect was the observed that caused lower recovery values at the studied spiking levels. However, for tomato, the recovery was 95% which might be due to the lower matrix effect.

Table (2) Retention times (RT's) and MS/MS conditions of the selected compounds

Pesticides	Quantifier MRM	Qualifier MRM	RT (min)
Acephate	184.1 > 143	184.1 > 125.1	0.925
Azinphos-ethyl	345.07 > 193.03	345.07 > 313.08	5.85
Azinphos-methyl	318 > 261	318 > 160	5.075
Bifenthrin	440 > 166	440 > 181	8.065
Chlorpyrifos	349.9 > 198	349.9 > 97	7.235
Chlorpyriphos-methyl	322.1 > 174.08	322.1 > 149.04	6.65
Coumaphos	363 > 307	363 > 289	6.355
Cyanophos	244.03 > 109.03	244.03 > 125.04	5.91
Cyfluthrin	451.1 > 191	453.1 > 193	7.51
Cyhalothrin_L	467.2 > 225	467.2 > 225	7.495

Cypermethrin	433 > 191	433 > 193	7.585
Deltamethrin	505.9 > 280.9	505.9 > 93.9	7.61
Diazinon	305.1 > 96.9	305.1 > 169	6.36
Dicrotophos	238 > 112	238 > 193	2.15
Dimethoate	229.07 > 201.02	229.07 > 121.07	2.565
Disulfoton-sulfone	307.1 > 97.1	307.1 > 153.1	4.67
Esfenvalerate	437.3 > 125	437.3 > 167	7.68
Ethion	385 > 199	385 > 97	7.17
Ethoprophos	243.2 > 131	243.2 > 97	5.91
Fenamiphos	304.1 > 202.1	304.1 > 217.1	6.09
Fenitrothion	278 > 79.1	278 > 109.1	5.68
Fenpropathrin	350.1 > 125	350.1 > 97	7.24
Fenthion	279.1 > 247.1	279.1 > 169.1	6.295
Fenvalerate	437.3 > 167	437.3 > 125	7.67
Isofenphos-methyl	332 > 289.9	332 > 272.9	7.6
Isofenphos-oxon	330 > 228.9	330 > 200.9	5.77
Malaoxon	315 > 98.9	315 > 127	4.125
Malathion	331 > 127	331 > 99	5.56
Methamidophos	142 > 124.9	142 > 93.9	0.81
Monocrotophos	224.1 > 98.1	224.1 > 127.1	1.865
Omethoate	214.1 > 183.1	214.1 > 125.1	1.03
Paraoxon ethyl	276.1 > 220.03	276.1 > 94.07	4.65
Paraoxon-methyl	248 > 202	248 > 90	3.52
Table (2) continued			
Parathion	291.9 > 110	291.9 > 236	6.145
Parathion-Methyl	263.9 > 109	263.9 > 79	5.265
Permethrin	408 > 183	410 > 183	7.895
Phenthoate	321 > 135	321 > 163	6.18
Phosalone	367.9 > 181.9	367.9 > 110.9	6.525
Phosphamidon	300.1 > 174.1	300.1 > 127.1	3.735
Phoxim	299 > 129	299 > 153	6.445
Pirimiphos-ethyl	334.1 > 182.1	334.1 > 198.1	7.06
Pirimiphos-methyl	306.1 > 108.1	306.1 > 164.1	6.505
Profenofos	372.9 > 302.6	372.9 > 127.9	6.94
Prothiophos	344.9 > 240.8	344.9 > 268.9	7.76
Pyrazophos	374 > 222.1	374 > 194	6.955
Pyrethrins	329.1> 177.03	329.1 > 279.13	7.465
Sulfotep	323 > 171	323 > 97	6.24
Tefluthrin	420 > 107	420 > 329	8.405
Tetrachlorvinphos	364.8 > 127	364.8 > 238.9	6.19
Tetramethrin	332.1 > 164	332.1 > 135	7
Triazophos	314.1 > 118.9	314.1 > 161.9	5.76

Table (3) Recoveries % and CV% of spiked samples at three levels calculated against matrix matched standard for Apple and Grape

Fruits Matrices	**Apple**				**Grape**			
Pesticides	SMX* %	**Rec. % ± C.V %**			SMX* %	**Rec. % ± C.V %**		
		10 µg/ kg	50 µg/ kg	100 µg/ kg		10 µg/ kg	50 µg/ kg	100 µg/ kg
Acephate	20	82 ±1	91 ±0.3	103 ±0.7	19	78 ±0.8	87 ±0.7	101 ±0.4
Azinphos-ethyl	101	110 ±8.3	84 ±5.2	84 ±6.3	91	74 ±14.9	81 ±8.6	83 ±4
Azinphos-methyl	97	103 ±4.5	94 ±2.2	94 ±1.5	92	93 ±3.1	98 ±3.2	98 ±2.5
Bifenthrin	29	92 ±13.9	83 ±0.9	84 ±1	76	64 ±14.2	69 ±7.9	65 ±3.5
Chlorpyrifos	89	102 ±3	89 ±0.8	92 ±1.8	112	118 ±6.9	95 ±2.6	92 ±1.2
Chlorpyriphos-methyl	82	90 ±2.8	89 ±1	94 ±1.1	94	98 ±1.4	100 ±2	102 ±2.1
Coumaphos	93	93 ±2.6	93 ±2.2	94 ±1.9	95	94 ±2.7	91 ±1.4	92 ±1.5
Cyanophos	98	96 ±4.3	94 ±1.6	94 ±2.2	97	91 ±4.5	95 ±2.4	96 ±2.4
Cyfluthrin	160	97 ±13	92 ±5.6	94 ±6.6	108	74 ±8.8	112 ±16.8	99 ±7.9
Cyhalothrin_L	180	86 ±14.6	81 ±6.3	84 ±7.4	103	94 ±7.6	104 ±4	114 ±2.1
Cypermethrin	100	72 ±12.9	77 ±2.7	85 ±4	98	73 ±14	83 ±9.7	108 ±2.9
Deltamethrin	122	90 ±16	79 ±3.8	83 ±3	99	88 ±6.4	98 ±4.7	106 ±0.9
Diazinon	100	96 ±3.2	90 ±1.3	90 ±1.9	97	98 ±3.1	95 ±0.8	94 ±2.6
Dicrotophos	74	112 ±15	114 ±12.7	102 ±13.7	92	109 ±2.1	108 ±1.2	104 ±1.2
Dimethoate	87	95 ±2.1	97 ±1.8	101 ±2.5	81	85 ±2.9	86 ±8.6	85 ±2.2
Disulfoton-sulfone	106	102 ±1.7	96 ±1.4	95 ±2.9	97	90 ±10.8	94 ±11.4	92 ±5.1
Esfenvalerate	86	113 ±15.	103 ±4.2	100 ±4.4	95	87 ±9	85 ±11	97 ±4.6
Ethion	80	91 ±1.2	92 ±0.9	94 ±2	92	93 ±3.5	93 ±2.3	95 ±0.2
Ethoprophos	105	94 ±2.5	92 ±1.1	89 ±1.1	100	100 ±2.3	99 ±1.4	96 ±1.3
Fenamiphos	110	101 ±7.1	82 ±4.1	78 ±4.8	101	105 ±1.8	98 ±2	98 ±1.1
Fenitrothion	96	101 ±8.7	94 ±3.6	97 ±1	92	109 ±4.3	104 ±3	108 ±1.7

Fenpropathrin	91	101 ±4.3	91 ±1.4	94 ±1.1	112	114 ±5.9	91 ±6.3	89 ±2.7
Fenthion	96	93 ±2.1	93 ±1.1	95 ±1.3	94	102 ±1.8	98 ±1	99 ±1
Fenvalerate	82	90 ±4.6	91 ±1.7	92 ±3.8	100	89 ±16.7	90 ±4.2	97 ±4.3
Isofenphosmethyl	94	112 ±3.6	95 ±1.9	93 ±3.8	95	100 ±2.6	100 ±1.4	99 ±2.1
Isofenphos-oxon	102	103 ±4	88 ±2.1	86 ±4.1	95	101 ±2.2	99 ±1.8	97 ±2.2
Malaoxon	72	100 ±16	112 ±5.1	117 ±9.3	89	101 ±3.5	103 ±2.4	106 ±1.8
Malathion	100	96 ±3.5	90 ±2.6	92 ±2.2	89	100 ±1.9	103 ±2.3	106 ±1.8
Methamidophos	15	80 ±1.1	88 ±0.6	71 ±0.3	16	90 ±0.5	79 ±0.5	83 ±0.4
Monocrotophos	34	94 ±0.8	101 ±0.7	113 ±1.7	33	92 ±0.7	91 ±1.5	95 ±0.8
Omethoate	24	95 ±0.9	88 ±0.4	95 ±0.7	23	99 ±0.4	92 ±0.7	99 ±0.5
Paraoxon-ethyl	118	91 ±0.6	101 ±0.9	78 ±1.8	95	80 ±17.7	98 ±15.6	77 ±4.8
Paraoxon-methyl	47	55 ±22.8	47 ±5.4	62 ±8.2	59	23 ±21.6	30 ±14.2	23 ±6.5
Parathion	101	78 ±8.6	83 ±4.4	85 ±3.4	94	104 ±5	97 ±2.1	95 ±1.8
Parathion-Methyl	96	97 ±5.9	92 ±2.8	94 ±2.5	95	99 ±5	95 ±2.7	99 ±1.5
Permethrin	67	76 ±13.2	76 ±1.1	79 ±1.8	101	70 ±4.1	77 ±7.5	75 ±7.8
Phenthoate	98	87 ±3.4	88 ±2.1	86 ±1.9	93	94 ±2.1	97 ±1.1	95 ±1.5
Phosalone	82	91 ±2.9	91 ±1.6	93 ±1.4	95	94 ±3.2	96 ±2	94 ±1.8
Phosphamidon	78	100 ±0.9	98 ±1.4	103 ±1.4	74	91 ±7.1	98 ±6.5	103 ±3
Phoxim	82	97 ±2.2	93 ±1.6	93 ±1.3	94	97 ±2.6	97 ±1.5	98 ±1.6
Pirimiphos-ethyl	96	94 ±2.8	90 ±2.4	88 ±1.4	103	97 ±1.7	97 ±1.2	93 ±1.4
Pirimiphos-methyl	119	105 ±16.	81 ±9.3	96 ±8.3	118	95 ±2.9	101 ±3.1	83 ±4.3
Profenofos	88	92 ±1.7	88 ±2.2	92 ±1.6	96	102 ±1.4	97 ±1.4	99 ±1.3
Prothiophos	46	82 ±4.5	77 ±3	78 ±2.1	103	75 ±14.1	83 ±6.6	88 ±4.8
Pyrazophos	86	102 ±11.	104 ±5.1	109 ±6.3	94	115 ±11.4	100 ±6	103 ±3
Table (3) continued								
Pyrethrins	99	86 ±3.7	87 ±2.5	90 ±2.8	101	83 ±9.8	88 ±5	96 ±2.1
Sulfotep	75	59 ±4.6	72 ±4	74 ±5.3	85	54 ±4.9	68 ±4.6	71 ±3.6
Tefluthrin	94	76 ±14.3	82 ±2.5	90 ±3.7	98	82 ±17	81 ±17	106 ±4
Tetrachlorvinphos	103	91 ±2.3	87 ±2.7	87 ±3.3	93	75 ±17.9	91 ±12.5	86 ±7.8
Tetramethrin	82	90 ±3.5	90 ±2.4	92 ±2.3	97	94 ±1.8	95 ±1.4	98 ±1.6
Triazophos	103	95 ±1.8	93 ±1.7	93 ±2	98	103 ±2.6	99 ±1.2	97 ±1

Table (4) Recoveries % and CV% of spiked samples at three levels calculated against matrix matched standard for Pepper and Tomato

Fruits Matrices	**Pepper**				**Tomato**			
Pesticides	SMX* %	**Rec. % ± C.V %**			SMX* %	**Rec. % ± C.V %**		
		10 µg/ kg	50 µg/ kg	100 µg/ kg		10 µg/ kg	50 µg/ kg	100 µg/ kg
Acephate	17	72 ±0.6	103 ±0.3	114 ±0.5	20	91 ±0.6	104 ±0.5	111 ±0.5
Azinphos-ethyl	63	70 ±12.7	78 ±6.8	73 ±6	81	109 ±3.1	108 ±3.1	112 ±3.3
Azinphos-methyl	77	80 ±3.9	84 ±1.6	84 ±2.9	93	107 ±3.3	105 ±1.4	106 ±2
Bifenthrin	27	24 ±28.2	41 ±2.6	40 ±4.5	44	168 ±4.3	186 ±0.4	193 ±0.7
Chlorpyrifos	87	88 ±4.7	75 ±2.4	77 ±1.1	91	99 ±1.8	107 ±0.5	109 ±0.9
Chlorpyriphos-methyl	74	118 ±8.9	107 ±7.2	98 ±5.1	97	92 ±1.6	109 ±1.6	102 ±1.2
Coumaphos	90	94 ±3	86 ±2.4	81 ±2.8	99	111 ±1.8	108 ±2.8	112 ±1.6
Cyanophos	89	72.7 ±5.5	78.3 ±8.8	81.9 ±6.1	99	110 ±3.5	100 ±1.5	102 ±1.9
Cyfluthrin	165	97.6 ±19.5	93.5 ±8.7	95 ±15	176	97 ±4.1	95 ±6.5	101 ±2.6
Cyhalothrin_L	108	102 ±7.1	99 ±3.1	96 ±3	136	97 ±4.1	95 ±6.5	101 ±2.6
Cypermethrin	105	89 ±6.9	95 ±3.2	94 ±1.6	82	112 ±7.8	107 ±1.9	114 ±1.6
Deltamethrin	104	86 ±11.1	91 ±4	92 ±2.4	106	109 ±8.6	108 ±3.8	109 ±1.2
Diazinon	75	118 ±6.7	103 ±7.3	83 ±6.1	93	101 ±2.8	103 ±2	105 ±1.8
Dicrotophos	84	75 ±6.1	82 ±6.9	73 ±4.7	139	87 ±14	103 ±15.5	94 ±16.4
Dimethoate	69	114 ±2.9	118 ±3.5	103 ±2.8	88	101 ±1.1	100 ±1.7	101 ±1.7
Disulfoton-sulfone	88	113 ±4.7	109 ±4.8	118 ±2.9	104	105 ±1.6	100 ±1.3	101 ±1.3
Esfenvalerate	88	108 ±12.9	112 ±7.2	114 ±2	80	90 ±7.4	94 ±2.3	100 ±2
Ethion	66	72 ±2.4	73 ±2.4	71 ±0.8	75	96 ±1.2	99 ±0.9	100 ±1
Ethoprophos	84	75 ±6.1	82 ±6.9	73 ±4.7	98	100 ±1.1	102 ±1.4	99 ±1.1
Fenamiphos	91	70 ±4.6	74 ±5.2	85 ±6.1	86	99 ±4.3	96 ±3.7	96 ±4.2
Fenitrothion	120	102 ±7.4	108 ±4.7	103 ±2.9	100	111 ±6.7	110 ±3.4	110 ±3.5
Fenpropathrin	82	97 ±8.4	81 ±3	86 ±1.3	95	100 ±2	108 ±0.8	112 ±0.7
Fenthion	76	70 ±4	72 ±5.9	75 ±4.2	96	103 ±1.4	102 ±1.4	105 ±1.5
Fenvalerate	92	99 ±6.9	115 ±4.8	119 ±2	85	111 ±5.4	117 ±3.8	112 ±2
Isofenphosmethyl	83	81 ±6.6	79 ±3.3	75 ±3.5	96	105 ±2.3	103 ±3.5	103 ±1.9
Isofenphos-oxon	84	79 ±3.6	79 ±2.3	74 ±3.4	90	105 ±2.3	103 ±3.5	103 ±1.9
Malaoxon	74	76 ±4.6	72 ±1.8	89 ±2.8	116	102 ±7.7	104 ±11.4	96 ±7
Malathion	74	77 ±4.6	72 ±1.8	90 ±2.8	91	98 ±1.4	103 ±2.4	105 ±0.4
Methamidophos	14	106 ±0.3	89 ±0.2	94 ±0.5	12	109 ±0.2	102 ±0.4	112 ±0.4
Monocrotophos	32	115 ±0.7	114 ±0.7	112 ±2.1	40	111 ±0.4	110 ±1	120 ±0.9
Omethoate	22	105 ±0.4	96 ±0.1	102 ±0.6	23	104 ±0.2	103 ±0.7	109 ±0.7

Paraoxon-ethyl	84	102 ±7	116 ±5.4	114 ±6.5	125	93 ±3	102 ±1.2	78 ±1.6
Paraoxon-methyl	30	34 ±10.2	62 ±11.2	46 ±14.8	97	92 ±5.6	115 ±7.9	117 ±6
Parathion	87	89 ±4.8	76 ±4.7	65 ±3.7	97	101 ±5	101 ±0.9	105 ±2
Parathion-Methyl	76	84 ±5.3	81 ±4.4	81 ±2.6	97	112 ±6.4	106 ±2.2	107 ±0.7
Permethrin	62	77 ±8.6	86 ±8.3	76 ±2.1	66	102 ±2.5	108 ±2.2	112 ±1.1
Table (4) continued								
Phenthoate	82	78 ±4.3	85 ±3.8	92 ±2.9	95	102 ±1.3	106 ±2.3	107 ±0.9
Phosalone	75	78 ±3	89 ±3.7	94 ±1.5	91	115 ±0.9	120 ±0.5	120 ±0.9
Phosphamidon	66	103 ±3.2	99 ±3	106 ±1.1	87	111 ±1.5	112 ±2	115 ±0.5
Phoxim	74	118 ±7.2	104 ±6.8	102 ±3.7	94	120 ±1.4	120 ±1.4	118 ±1.2
Pirimiphos-ethyl	101	93 ±2.6	92 ±2.5	87 ±1.3	93	107 ±1.2	108 ±1.2	105 ±1.4
Pirimiphos-methyl	102	89 ±4.7	94 ±6.1	73 ±12.7	93	95 ±7.8	110 ±5.8	94 ±1.2
Profenofos	88	89 ±1.4	84 ±3.3	79 ±1.2	89	114 ±0.9	111 ±0.7	112 ±1.1
Prothiophos	91	82 ±6	97 ±3.9	102 ±2.1	71	114 ±1.7	109 ±1	110 ±1.2
Pyrazophos	124	108 ±11.8	109 ±10.2	106 ±3.5	94	107 ±16.8	103 ±10.6	108 ±1.9
Pyrethrins	98	109 ±3.2	111 ±2.4	111 ±0.9	81	82 ±3	95 ±1.4	106 ±1.1
Sulfotep	59	35 ±8.6	46 ±5.7	40 ±4.1	78	95 ±2.1	114 ±6.2	120 ±0.9
Tefluthrin	97	92 ±6.7	97 ±3.1	97 ±2.2	88	112 ±7.8	107 ±1.9	114 ±1.6
Tetrachlorvinphos	72	75 ±10.9	75 ±10.5	74 ±3.5	91	106 ±1	106 ±2.9	108 ±1.5
Tetramethrin	97	101 ±3.8	98 ±1.2	96 ±2.3	93	115 ±0.2	118 ±1.6	120 ±1.3
Triazophos	92	88 ±1.7	86 ±2.2	80 ±1.6	102	104 ±1.2	104 ±1.6	103 ±2.3

*Where SMX: Standard matrix against standard in solvent %, CV%: Coefficient of variation

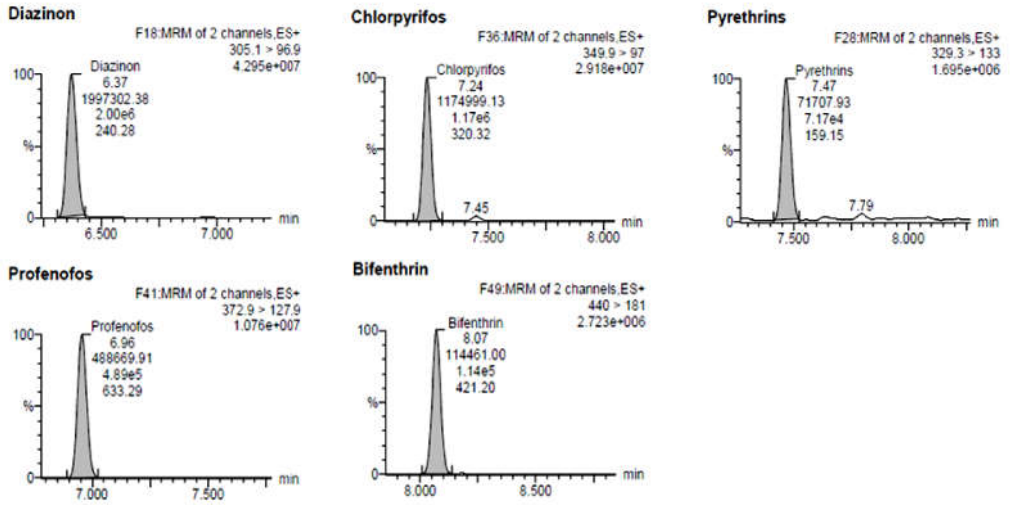

Fig.1 LC-MS/MS Chromatograms for determination of Apple sample spiked with pesticides at a concentration of 50 µg L^{-1}

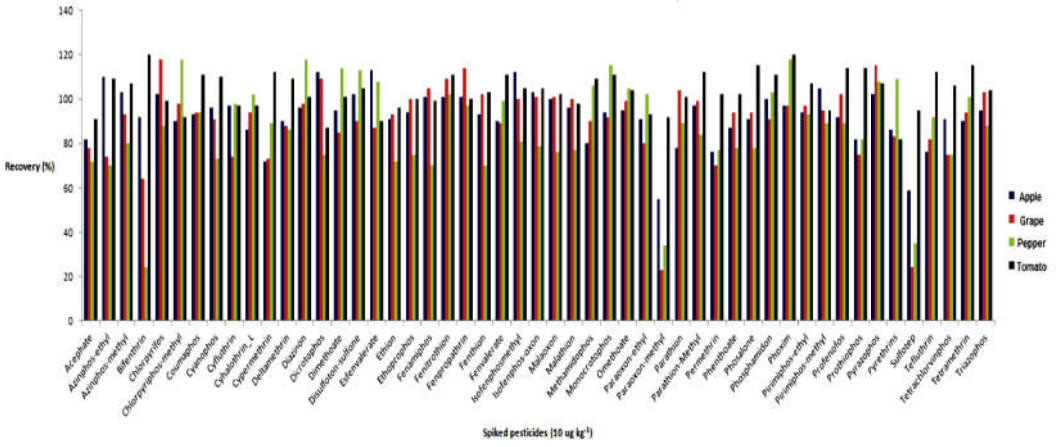

Fig.2 Comparison of the recovery (%) of the measured fruits and vegetable samples spiked with pesticides at a concentration of 50 µg L^{-1}

Matrix effect

The complex matrix of fruit and vegetable samples can greatly affect the analyte signals, an enhancement of the background noises, and suppression in the analyte responses. These undesirable effects are related to the concentrations and the protonation level of the extracted components. Thereby, matrix effects may

result in either positive or negative responses. Usually, for liquid separation followed by tandem mass, we can find only suppression effect which was in agreement of Verzegnassi *et al* [17].

The obtained data of injection of standard on matrix illustrated that, all studied matrix caused suppression more than 50% for each of, acephate, bifenthrin (except grape), methamidophos, monocrotophos,omethoate, paraoxon-methyl (except tomato) at the mentioned operating conditions of LC-MSMS.

Table 3. In order to determine the matrix effect; calibration curves were constructed using a matrix matched standard. The results were compared to those of the standard calibration curves. The recoveries of spiked blank samples were calculated using one point matrix matched standard.

Method validation results

Validation was carried out according to SANCO guideline 12459/2011 (SANCO). [18] Four matrices have been selected namely apple, grapes, green pepper, and tomato using 6 replicate measurements at 3 levels of 10, 50 and 100 µg kg^{-1} covering validation requirement such as LOQ (100 µg kg^{-1}) and LOD (sensitivity). The recovery (%) range of method varied from 70 to 120%. The uncertainty (Expanded Uncertainty) for the method was 39.2% and all parameters were calculated using recovery% and RSD%. The following equation is used for combined uncertainty calculations; according to Prudnikov [19]**.**

$$U_C = \sqrt{(U_{precision})^2 + (U_{sp}) + (U_{\mathrm{Re}c})^2 + +U_{\mathrm{Re}f}}$$

CONCLUSION

This optimized method using acetonitrile extraction followed by tandem mass spectrometry determination is simple, rapid and reliable. Satisfactory recoveries and repeatability were observed. The described method requires little amount of solvents, sample and detection time (10 min) and could be used in controlling levels of pesticides from different classes in fruits and vegetable samples.

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