International Journal of Secondary Metabolite
2018, Vol. 5, No. 2, 130–139

DOI: 10.21448/ijsm.408174

Published at http://www.ijate.net http://dergipark.gov.tr/ijsm Research Article

Development and validation of modified QuEChERS method coupled with GC-MS/MS for 123 pesticide residues in food

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Abstract: In this study, a gas chromatography-tandem mass spectrometry (GC-MS/MS) instrument, which has been widely used in recent years and has high separation power, selectivity and ability to identify pesticides has been used. It is aimed that the main criterion of this analytical method, in which the QuEChERS methodology is used, is applicable to fast, easy, cheap, environmentally friendly and different matrices. At the same time with this method, 123 pesticide residues and their degradation products were quantitatively assayed by GC-MS/MS as well as method validations in tomatoe, lemon, lettuce, almonds, raisins, honey, green pepper, milk and flour. Tomatoe was selected as potential reference matrixes for the target. The steps of concentration and solvent exchange were performed in the resultant extracts for the purpose of improving analytical performance in terms of recovery, precision, linearity, of reducing the amount of co-extracts. Multiple reaction monitoring (MRM) was used to identify and quantify the pesticides. The samples were extracted with 1% acetic acid in acetonitrile, anhydrous magnesium acetate, anhydrous magnesium sulfate and clearing agent. For all pesticides, good linear calibrations with coefficients (R2) ≥0.99 for nearly all of the analytes were obtained. Limit of quantitation of most of the pesticides were in the range of 5-10 ng/g, and recovery of the method validation accuracy parameter was done at two different concentrations 10 ng/g and 50 ng/g were 88.6 - 99.7% and CV 1.60 – 14.0%.

ARTICLE HISTORY

Received: 25 December 2017
Revised: 19 March 2018
Accepted: 23 March 2018

KEYWORDS

Pesticide
Chlorpyrifos
Deltamethrin
Validation
GC-MS/MS

1. INTRODUCTION

Every kind of chemical compound used to protect agricultural products from disease, harmful and foreign weeds is called pesticide. A pesticide is known to be any compound or mixture of compounds that prevents, removes or protects from the spread of any unwanted organism (pest). The usage of pesticides in agriculture after World War II, the world has multiplied to increase food production. Since then, the development of different types of pesticides belonging to various groups has become important [1].

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ISSN-e: 2148-6905 /© IJSM 2018

As a result of producers' unconscious and excessive use of pesticides, resistant populations are formed, natural enemies are adversely affected and harmful effects occur in terms of environment and human health. Regular use of pesticides is detrimental to the ecosystem. Many international organizations and countries are extremely worried about pesticide residues. The pesticide maximum residue limit (MRL) determination [2,3] is being undertaken to protect public health and ensure food safety. Numerous studies have been carried out naturally on the simultaneous determination of analytical techniques, especially multiple residues, in the detection of pesticide residues [4,5]. This study is quite up to date on these aspects.

Various extraction methods have been stated in the literature including hydrolysis with chloric or sulfuric acid, soxhlet extraction, liquid-liquid extraction (LLE), extraction with organic solvent directly from the solid matrix, and more recently solid-phase microextraction (SPME) [6-10]. Purification might be needed to remove co-eluted matrix material and reduce analytical background noise. To date, purification techniques usually included elution of sample extracts with hexane and dichloromethane on chromatographic columns packed with acidified silica gel, deactivated alumina or florisil [11,12]. Recently, an attempt was made to replace these laborintensive clean-up steps by LLE or purification on solid-phase extraction (SPE) cartridges [10]. Different extraction and measurement methods have been used by various scientists for the detection of multiple classes of pesticides in many food, vegetables and fruit varieties [13,14].

In 2003, Anastassiades et al. [15] developed a fast, easy, inexpensive, effective, robust and secure (QuEChERS) method to overcome the critical deficiencies and practical limitations of existing methods. Then, Lehotay et al. 2010 developed a sample preparation method with the citrate-buffered QuEChERS procedure [16]. The main criterion for choosing any methodology is that analytical method is applicable to fast, easy, inexpensive and different matrices.

Developing a new methodology requires the resolution of a large number of problems. Like extraction solvent selection. Solvents used to identify pesticide residues in food matrices [17-19], and these solvents provide high analytical recovery. Acetone can be mixed with water, but it is not possible to separate water from the solvent without using apolar solvents. On the other hand, it is the part where ethyl acetate is mixed with water. It causes unnecessary addition of apolar solvents to separate water, but most of the highly polarized pesticides are not separated. The acetonitrile extracts of foods contain less interfering substances than ethyl acetate and acetone extracts and acetonitrile can be separated from the water quite easily (salt precipitation), which is the preferred extraction solvent of acetonitrile methodologies. The aim of the study is to develop and validate the modified QuEChERS method for 123 pesticide residue in food using GC-MS/MS.

2. MATERIAL AND METHODS

2.1. Reagent and Materials

Pesticide standards were purchased from Dr. Ehrenstorfer. Acetonitrile, methanol, acetic acid, anhydrous sodium acetate, ammonium formate, anhydrous magnesium sulphate (98% purity), Silica gel 60, PSA (Primary Secondary Amine) sorbent (40 µm particle size) were purchased from local supplier. HPLC-grade water (18.2 mΩ) was purified using a Millipore Elix Advantage 10 and Milli-Q Advantage A10 system that comprise reverse osmosis, ion exchange, and filtration steps.

The samples used in the study (tomatoes, lemons, lettuce, almonds, raisins, honey, green pepper, milk and flour) were supplied without pesticides from Fethiye, Datça, Marmaris. Fruits

and vegetables were purchased from producers of organic farming. This study has been selected as representative matrices by considering the directive of the European Union.

2.2. Pesticide Standards Main Stock Solution

All pesticide standard substances Dr. Ehrenstorfer are certified reference materials are taken from local supplier. The pesticide standards were weighed in a 50 mL volumetric flask as approximately 10 mg with a precision of 0.01 mg. The volume was completed with methanol. The main stock solutions are stored at -18 °C.

2.3. Pesticide Mix Solutions

The pesticide was withdrawn with an automatic pipette such that the concentration of each of the standard main stock solutions was 1 mg L^{-1} (1 ppm). The pesticide mix solutions for GC-MS/MS were prepared separately in methanol. The pesticide mixture solutions were stored at 4 °C.

2.4. Pesticide Working Solutions

To determine the GC-MS/MS conditions of the pesticides, the concentrations from the parent stock solutions were set to be about 500 ng mL-1 (ppb). The target ion, qualifier ions, collision energies and retention time (Rt) were determined separately in the device. For the method-device optimization, the acquisition method was created by dividing it into eight time segments.

2.5. Method

GC-MS/MS instrument coupled with Agilent Technologies 7890A GC gas chromatography 7000B Triple Quadrupole mass spectrometer was used in the study. Agilent Technologies 7693 autosampler and multi-mode inlet system were used in the system. This method involves extraction of the extract using acetonitrile pre-extraction and separating solid phase extraction. For liquid-liquid separation, salt precipitation is carried out using anhydrous magnesium sulfate, so that the water is separated from the sample.

Most of the methods applied worldwide at the clean-up level use Primary Secondary Amine (PSA) in most cases. PSA is an expensive chemical that significantly increases the cost of analysis. In this study, the sample extracts have been studied using silica-gel, which are cheaper, simple and effective chemicals, with GC-MS/MS to detect multiple pesticide residues.

The most common approach to derive from the matrix effect is to use matrix-match calibration standards [20,21]. However, a large number of blank matrices are required to work with matrix-match, which requires extra extraction. In this study, a separate matrix-match calibration curve is used for each matrix. Thus, it was already known how each pesticide will behave when interacting with the matrix.

Approximately 1 kg of the sample is disintegrated and homogenized with the aid of the sample shredder. Fifteen grams of the homogenized sample is transferred to a 50 mL falcon tupe. Add 15 mL 1% acetic acid in acetonitrile. It is vigorously shaken for 1.5 min. After addition of 1.5 g of anhydrous magnesium acetate and 6 g of anhydrous magnesium sulfate, the mixture is shaken vigorously for 1.5 min and 150 µL (5 ng mL^{-1}) of Internal Standard Solution is added. Centrifuge at 4000 rpm for 5 min. Then transfer to 8 mL of extraction tubes then add 400 mg of clearing agent (Silica-gel, PSA, C18 or GCB) and 1200 mg of anhydrous magnesium sulfate, shake, centrifuge. The extract is transferred to 2 mL vials and 50 µL 3-phenyl phosphate (TPP) is added, for the analysis using GC-MS/MS.

2.6. Instrument and Pesticide Optimization

In this study, the parent ion, daughter ions, cone voltage and collision energies and retention times (Rt) of each pesticide were determined separately in the device. Multiple

reaction monitoring (MRMs) were generated for the analysis method. The process method was established in which the ions of each pesticide were written at the time of retention. Retention time, parent and precursor ions, collision voltages for each of the pesticide were identified in order to obtain maximum signal. Standard main stock solutions were prepared in the concentration of 50-500 ng mL^{-1} and precursor and product ions of each the pesticide were determined in GC-MS/MS using SCAN mode. Ions were examined in the detected peak, and compared with molecular weight of each pesticide. It is examined by looking at the structure of the molecule, whether it is equal to the molecular weight or fragmented from the molecule. In order to detect target ion and breakdown ions in the MRM mode, different impact energy is tried in increments of 5 V between 5 and 30 for each transition of the analytes to detect the collision energy and the collision energy producing the highest area was detected. An example of this work is illustrated below for propham in Figure 1.

The pesticide representative, propham, three transitions were examined. Three different collision energies were tried, 10V, 20V, 30V. First is 179.1 > 92.2 *m/z*. The highest peak area was detected as 3.209 abundance at 20 V. Second is 93.0 > 66.0 *m/z*. The highest peak area was detected as 198.532 abundance at 20 V. With this study, for propham; 93 > 66 *m/z* and 93> 65 *m/z* transitions and the result of the collision energy optimization study have been determined.

2.7. Method Optimization

Tomato was used as the first matrix in the method optimization study. Four parallel runs according to the sample preparation procedure. The specimens were run separately for GC-MS/MS and the spikes at 50 ng g^{-1} were made in parallel from GC mixtures. Approximately 1 kg sample was crushed and homogenized with a chopper. A 15-g portion of the homogenized sample was weighed in an analytical balance with a 50 mL falcon tube and 150 µL 5 ppm di ethatyl ethyl (DEE) (internal standard) and 750 µL 1 ppm pesticide solution were added. 15 mL of acetonitrile containing 1% acetic acid was added. It was vigorously shaken by hand for 1.5 min. The previously weighed 1.5 g anhydrous sodium acetate and 6 g anhydrous. Magnesium sulfate was added, then vigorously shaken again manually for 1.5 min and centrifuged for 5 min at 4000 rpm. Previously, a 15 mL centrifuge tube was prepared by weighing 400 mg of cleaning agent (PSA/Silica gel) and 1200 mg of anhydrous magnesium sulfate, and after centrifugation, 8 mL of the supernatant was transferred, then shaken by hand and centrifuged at 4000 rpm for 5 min. 1 mL of the supernatant was transferred to 2 mL vials and 50 µL TPP was added. The tube was agitated in the mixer. The vial was analyzed in GC-MS/MS.

3. RESULTS AND DISCUSSION

In this study, all of the analyzed pesticide analytes could be successfully chromatographically separated using an Agilent Technologies 7890A GC gas chromatography and HP-5MS UI (5% phenyl methylsiloxane) 15 m x 250 µm x 0.25 µm capillary column and oven temperature program in MRM mode. The oven program was established in GC for chromatographic separation of analytes. Multiple reaction monitoring (MRM) was used to identify and quantify the pesticides with a precursor ion and at least two product ions in Agilent Technologies 7000B Triple Quadrupole MS/MS. Firstly, the instrument method optimization was accomplished. The pesticide standard working solutions were prepared separately for each of the analte, and then tested to determine the precursor and product ions, collision energies retention time in GC-MS/MS with scan mode. Ions were evaluated in detected peak by comparing its molecular weight. The collision energies were detected for the each of transision in the range of 5-30 V by increments of 5V. The parameters of GC-MS/MS and data related to method validation were displayed in Table 1.

Table 1. GC-MS/MS instrument and the pesticide method validation parameters

No	Pesticide	Rt min	Target ion *m/z*	Product ion *m/z*	Dwell time sec	Collision energy V	Average recovery %	S	v	CV%	n
1	Acrinathrin	15.39	181.1	152.1, 127.1	20	25, 30	95.5	4.44	19.69	4.65	120
2	Alachlor	8.50	188.1	160.1, 130.1	25	10, 42	94.5	2.40	5.75	2.54	119
3	Aldrin	9.25	263.0	193.0, 191.0	25	30, 30	94.6	4.07	16.54	4.30	120
4	Allethrin	10.89	123.1	81.1, 79.1	20	10, 20	98.5	3.73	13.95	3.79	120
5	Azinphos-ethyl	15.32	132.0	77.0, 132.0	20	12, 1	88.6	6.83	46.68	7.71	120
6	Azinphos-methyl	14.83	160.1	132.1, 77.1	20	5, 20	89.1	7.29	53.21	8.19	106
7	Benfluralin	5.86	292.1	264.0, 160.1	10	20, 15	91.6	3.85	14.81	4.20	120
8	Bifenthrin	14.44	181.1	166.1, 165.1	20	15, 30	96.4	3.29	10.83	3.42	120
9	Bromocyclen	7.59	358.7	278.0, 243.0	20	5, 20	94.3	2.63	6.94	2.79	120
10	Bromophos-ethyl	11.26	358.7	331.0, 303.0	20	5, 15	96.9	2.71	7.35	2.80	120
11	Bromopropylate	14.34	183.0	155.0. 76.0	20	15, 35	96.8	3.66	13.40	3.78	120
12	Captafol	4.15	79.0	77.0, 78.9	10	20, 20	96.6	3.14	9.86	3.25	119
13	Carbophenothion	13.33	342.0	96.9, 157.0	20	10, 10	93.7	3.10	9.63	3.31	120
14	Chinomethionate	10.94	234.0	148.0, 206.0	20	17, 9	89.1	12.48	155.68	14.01	120
15	Chlorbenside	10.91	125.0	99.0, 89.0	20	20, 20	93.7	1.84	3.40	1.97	120
16	Chlordane, cis-	11.01	372.7	266.1, 264.1	20	25, 25	96.1	2.40	5.77	2.50	120
17	Chlordane, trans-	11.41	372.7	266.1, 264.1	20	25, 25	96.1	2.40	5.77	2.50	120
18	Chlorfenapyr	12.64	247.0	227.0, 59.0	15	15, 10	90.9	4.86	23.60	5.34	119
19	Chlorfenprop-methyl	4.98	195.6	164.6, 101.8	10	15, 35	93.8	2.64	6.98	2.82	120
20	Chlorfenson	11.65	301.8	174.8, 111.1	15	5, 22	96.6	2.58	6.65	2.67	120
21	Chlorfenvinphos	10.80	267.0	159.0, 81.0	20	20, 40	94.5	4.22	17.78	4.46	120
22	Chlorobenzilate	12.72	139.0	111.0, 75.0	15	15, 30	95.3	2.98	8.87	3.13	120
23	Chloroneb	4.31	191.0	141.0, 113.0	10	10, 15	99.6	2.21	4.89	2.22	120
24	Chlorothalonil	7.40	265.9	230.9, 133.0	20	20, 40	92.5	5.57	31.08	6.03	120
25	Chlorpropham	5.54	213.0	171.0, 127.0	10	5, 10	97.0	4.77	22.73	4.91	120
26	Chlorpyrifos	9.62	196.9	168.9, 107.0	25	16, 44	93.7	2.51	6.32	2.68	120
27	Chlorpyrifos methyl	8.29	286.0	270.9, 93.0	25	20, 25	93.5	2.86	8.16	3.06	120
28	Chlorthal-dimethyl	9.72	299.0	221.0, 223.0	25	25, 25	98.8	2.49	6.20	2.52	120
29	Chlorthiamid	8.03	170.6	135.6, 99.7	25	15, 35	92.5	4.69	22.02	5.07	120
30	Chlozolinate	10.18	259.0	147.1, 188.0	25	15, 10	89.5	2.92	8.51	3.26	120
31	Cyanophos	6.88	242.5	124.8, 108.8	20	15, 15	91.2	2.40	5.76	2.63	120
32	Cyfluthrin	16.24	163.0	127.1, 91.1	20	5, 15	96.8	4.19	17.54	4.33	120
33	Cyhalothrin (I, II, III, IV)	15.22	197.0	171.0, 161.0	20	15, 10	90.5	4.75	22.61	5.25	118
34	Cyhalothrin, λ-	15.22	181.1	152.1, 127.1	20	29, 33	94.2	4.25	18.04	4.51	120
35	Cypermethrin	16.60	181.1	152.1, 127.1	20	27, 33	92.6	3.93	15.48	4.25	119
36	Dazomet	6.24	161.8	88.9, 72.9	10	5, 40	95.0	5.31	28.16	5.59	120
37	3,4- Dichloraniline	3.83	160.5	125.8, 89.9	10	15, 25	99.5	3.17	10.02	3.18	118
38	3,5- Dichloraniline	3.67	160.7	98.8, 89.9	10	25, 25	99.6	2.52	6.34	2.53	120
39	DDD, o,p'-	12.18	235.0	199.1, 165.1	15	15, 30	98.4	2.01	4.03	2.04	120
40	DDD, p,p'-	12.84	235.0	199.1, 165.1	15	20, 25	96.1	2.19	4.81	2.28	120
41	DDE, o,p'-	11.24	246.0	211.0, 176.1	20	20, 40	96.5	2.27	5.17	2.36	120
42	DDE, p,p'-	12.01	246.0	176.1, 175.1	15	40, 40	95.7	2.46	6.08	2.57	120
43	DDT, o,p'-	12.84	235.0	199.1, 165.1	15	20, 20	93.8	2.23	4.99	2.38	120
44	DDT, p,p'-	13.49	235.0	199.1, 165.1	20	20, 30	89.5	4.79	22.98	5.36	118
45	Deltamethrin	17.79	253.0	174.0, 93.0	20	6, 22	91.5	5.00	24.99	5.46	117
46	Demeton-S-methyl	5.25	88.1	60.0, 59.0	10	5, 20	92.2	4.15	17.25	4.50	120
47	Dibromobenzophenone, 4,4	12.29	339.9	185.0, 182.9	15	22, 21	98.0	2.24	5.01	2.28	120
48	Dichlobenil	3.40	171.0	136.0, 100.0	10	30, 15	97.2	2.36	5.57	2.43	120
49	Dichlofenthion	8.08	279.0	223.0, 205.0	25	10, 25	96.0	2.53	6.39	2.63	120
50	Dichlorobenzophenone, 4,4	9.60	249.9	214.9, 139.0	25	11, 10	97.2	2.23	4.98	2.30	120
51	Diclofop-methyl	13.84	339.3	252.4, 183.5	20	15, 35	91.2	4.85	23.55	5.32	120
52	Dicloran	6.28	206.0	176.0, 123.9	10	10, 25	93.7	3.24	10.47	3.45	120
53	Dieldrin	11.93	263.0	193.0, 191.0	15	30, 30	96.1	3.22	10.34	3.35	120
54	Dinitramin	7.39	304.6	260.6, 243.6	20	5, 5	97.7	5.86	34.35	6.00	108

No	Pesticide	Rt min	Target ion *m/z*	Product ion *m/z*	Dwell time sec	Collision energy V	Average recovery %	S	v	CV%	n
55	Diphenylamine	5.26	169.0	167.0, 168.0	10	20, 15	97.2	2.36	5.58	2.43	120
56	Disulfoton	7.27	88.1	60.0, 59.0	20	5, 25	91.1	2.59	6.70	2.84	120
57	Endosulfan sulfate	14.93	271.9	236.9, 116.9	20	16, 44	99.7	1.94	3.78	1.95	120
58	Endosulfan, α-	11.31	240.8	205.9, 136.0	20	16, 40	97.1	3.07	9.45	3.17	118
59	Endosulfan, β-	12.58	195.0	159.0, 125.0	15	9, 28	95.6	3.67	13.43	3.84	120
60	Endosulfan-sulphate	13.38	271.9	236.9, 116.9	20	16, 44	97.5	2.03	4.11	2.08	120
61	Endrin	12.38	263.0	193.0, 191.0	15	30, 30	95.3	3.36	11.27	3.52	120
62	Esfenvalerate	17.23	125.0	99.2, 89.1	20	25, 25	91.7	3.52	12.40	3.84	120
63	Ethion	13.01	231.0	175.0, 129.0	15	24, 10	92.7	3.51	12.29	3.78	120
64	Etridiazole	3.98	183.0	139.9, 108.0	10	20, 40	93.7	5.76	33.14	6.15	118
65	Etrimfos	7.58	292.0	181.0, 153.0	20	5, 20	95.1	3.53	12.48	3.72	120
66	Fenchlorphos	8.65	284.9	269.9, 93.0	25	15, 25	94.7	2.80	7.81	2.95	120
67	Fenitrothion	9.04	277.0	260.0, 109.0	25	4, 20	89.3	3.60	12.98	4.03	120
68	Fenson	9.83	141.0	77.1, 77.0	25	15, 20	94.1	2.10	4.39	2.23	120
69	Fenvalerate (I-II)	17.23	167.0	125.0, 89.1	20	10, 40	90.8	4.09	16.72	4.50	120
70	Fipronil	10.91	367.0	228.0, 213.0	20	30, 30	91.6	3.29	10.82	3.59	120
71	Fluchloralin	7.32	306.0	264.0, 206.0	20	15, 15	94.2	4.11	16.92	4.37	120
72	Flucythrinate (I-II)	16.61	199.0	157.0, 107.0	20	15, 5	92.4	4.06	16.52	4.40	120
73	Flumethrin	5.86	215.5	158.7, 76.9	10	25, 25	96.6	5.15	26.48	5.33	120
74	Fluvalinate-τ (I-II)	17.45	250.0	199.9, 54.9	20	23, 20	94.2	5.80	33.66	6.16	118
75	Formothion	7.62	170.0	93.0, 63.0	20	10, 25	94.1	7.30	53.34	7.76	115
76	Furalaxyl	10.97	241.6	94.9, 151.7	20	15, 12	93.1	2.77	7.69	2.98	113
77	Halfenprox	16.45	477.3	237.0, 171.0	20	10, 20	89.6	3.68	13.58	4.11	120
78	HCH, α-	6.04	181.0	145.0, 109.0	10	15, 30	95.4	2.30	5.29	2.41	120
79	HCH, β-	6.60	181.0	145.0, 109.0	20	15, 30	96.3	2.04	4.18	2.12	120
80	HCH, γ- (Lindane)	6.71	181.0	145.0, 109.0	20	15, 30	95.2	2.25	5.08	2.37	120
81	HCH, δ-	7.26	181.0	145.0, 109.0	20	15, 30	94.1	2.36	5.56	2.51	120
82	Heptachlor	8.39	271.9	236.8, 142.9	25	25, 40	92.0	2.73	7.48	2.97	117
83	Heptachlor endo-epoxide	10.47	183.0	154.9, 118.9	25	15, 30	95.4	3.59	12.89	3.76	120
84	Heptachlor exo-epoxide	10.35	352.9	281.9, 262.8	25	20, 25	96.8	2.45	6.03	2.54	118
85	Mirex	14.93	272.0	235.0, 216.9	20	25, 20	98.5	1.76	3.11	1.79	120
86	Nitralin	14.10	303.0	302.0, 145.0	20	10, 26	88.8	7.43	55.19	8.37	116
87	Nitrapyrin	3.97	193.8	167.0, 158.0	10	20, 20	95.6	4.92	24.25	5.15	120
88	Nitrofen	12.43	282.9	253.0, 202.1	15	10, 25	89.5	4.05	16.41	4.52	120
89	Nitrothal-isopropyl	9.93	236.1	194.1, 148.1	25	5, 20	92.8	3.17	10.04	3.42	120
90	2-phenylphenol	4.39	170.0	141.0, 115.0	10	15, 35	96.5	1.55	2.41	1.61	120
91	Parathion (-ethyl)	9.63	291.0	109.0, 81.0	25	10, 35	91.0	3.26	10.66	3.59	120
92	Parathion methyl	8.29	263.0	109.0, 79.0	25	12, 33	91.1	3.10	9.61	3.40	120
93	Pentachloroaniline	7.76	265.0	230.0, 194.0	25	10, 20	96.5	2.67	7.14	2.77	120
94	Pentachloroanisole	6.29	264.8	143.0, 117.1	10	35, 35	95.3	3.11	9.65	3.26	120
95	Permethrin	15.82	183.1	168.1, 153.1	20	14, 16	95.0	3.66	13.38	3.85	120
96	Perthane	12.57	222.8	179.2, 165.3	15	20, 25	94.5	3.28	10.78	3.48	120
97	Phorate	5.96	231.0	174.9, 128.9	10	10, 25	95.2	4.18	17.50	4.39	120
98	Phosmet	14.85	160.0	133.0, 77.1	20	15, 30	95.6	7.20	51.91	7.54	116
99	Phthalimide (Folpet)	4.00	147.0	76.0, 103.0	10	30, 6	95.2	3.35	11.21	3.52	120
100	Procymidone	10.98	283.0	96.1, 67.1	20	10, 39	98.4	2.85	8.15	2.90	120
101	Profluralin	7.01	318.1	284.1, 199.1	20	10, 15	89.9	2.78	7.74	3.10	120
102	Propham	3.96	93.0	66.0, 65.0	10	15, 25	102.7	2.45	6.02	2.39	120
103	Prothiophos	11.87	266.5	238.5, 240.6	15	5, 5	94.9	2.73	7.43	2.87	120
104	Pyraflufen-ethyl	13.72	349.0	307.0, 349.0	20	10, 10	96.3	4.11	16.91	4.27	120
105	Pyrimidifen	17.05	160.6	134.8, 90.9	20	15, 35	94.4	3.18	10.08	3.36	116
106	Quinalphos	10.84	146.1	118.1, 91.1	20	10, 30	95.0	2.76	7.60	2.90	120
107	Quintozene	6.83	236.9	142.9, 118.9	20	30, 25	91.8	2.93	8.61	3.20	120
108	Resmethrin	14.01	123.1	95.1, 81.1	20	5, 5	97.5	4.95	24.54	5.08	120
109	S421	8.66	130.0	130.0, 95.0	25	5, 20	93.7	3.54	12.54	3.78	120

No	Pesticide	Rt min	Target ion *m/z*	Product ion *m/z*	Dwell time sec	Collision energy V	Average recovery %	S	v	CV%	n
110	Spiromesifen	14.18	272.0	254.0, 209.0	20	5, 20	93.5	5.86	34.39	6.27	120
111	Sulprofos	13.19	322.1	155.9, 97.0	20	5, 25	93.0	3.49	12.18	3.75	120
112	Tecnazene	5.12	202.9	142.9, 83.0	10	20, 25	92.0	3.21	10.33	3.49	120
113	Tefluthrin	7.54	177.0	137.0, 127.0	20	15, 15	95.7	2.00	3.99	2.09	120
114	Terbacil	7.33	160.7	143.8, 116.9	20	15, 5	94.5	4.79	22.95	5.07	120
115	Terbufos	6.88	231.0	174.9, 128.9	20	10, 25	92.0	3.79	14.36	4.12	120
116	Tetrachlorvinphos	11.49	329.0	108.9, 93.0	15	20, 10	96.3	5.08	25.77	5.27	120
117	Tetradifon	14.71	355.8	159.0, 353.9	20	12, 5	98.0	3.32	10.99	3.38	120
118	Tetrahydrophthalimide	4.14	151.0	122.0, 79.0	10	11, 19	98.0	2.93	8.56	2.99	120
119	Tetrasul	13.03	251.8	216.9, 182.2	20	25, 25	95.9	2.64	6.95	2.75	120
120	Thiometon	6.17	125.0	79.0, 47.0	10	20, 10	93.4	2.98	8.90	3.19	120
121	Tolclofos-methyl	8.40	265.0	250.0, 93.0	25	15, 25	97.6	2.32	5.38	2.38	120
122	Trifluralin	5.81	306.1	264.0, 160.0	10	5, 26	92.2	3.36	11.26	3.64	120
123	Vinclozolin	8.31	212.0	145.0, 109.0	25	25, 40	96.1	2.54	6.45	2.64	120

Rt: retention time; S: standard deviation; v: variance; CV: coefficient of variance; n: number of samples

Tomatoes, lemons, lettuce, almonds, raisins and honey were selected as representing food matrix for the validation of the method performance. The pesticide matrix solutions (tomato, lemon, lettuce, almond, raisins and honey) were made ten repetitions for three different days for each level at two different concentrations, with concentrations of 10 ng mL^{-1} and 50 ng mL^{-1} for each analyte.

The specificity parameter of an assay is a measure of the extent to which the method can determine a particular compound in the analyzed matrices without interference from matrix components. The validation procedure should confirm the ability of the method to unequivocally assess the analyte in the presence of other components that may be present (for example, impurities, degradation products and matrix components). The chromatographic separation of all analytes from each other was accomplished successfully by an Agilent Technologies 7890A GC gas chromatography and HP-5MS 5% phenyl methylsiloxane (15 m x 250 µm x 0.25 µm) the capillary column, oven program in MRM mode.

The selectivity is that a method analyzes a given compound without interfering with the matrix components in the matrix. It is accomplished by Agilent Technologies 7890A GC gas chromatography 7000B MS/MS Triple Quadrupole GC-MS/MS system. The identification, validation, calculation of an analyte takes place with at least one precursor ion and at least two product ions in the MRM mode.

The limit of detection (LOD) is the limit lowest residue concentration that result could not submit. The limit of quantitation (LOQ) is the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met [22]. In the European Commission Regulation (EC/299) [23], maximum residue limits (MRLs) are specified using LOQ values.

With the method validation studies, the LOD - LOQ parameter was used in all studied matrices. In the repeatability study of the analysis, the recovery of the method validation accuracy parameter was done at two different concentrations, 10 ng g^{-1} and 50 ng g^{-1}, with percent recovery and % CV values. The recovery results obtained in GC-MS/MS for contaminants for authenticity and precision subparameters were 88.6 - 99.7% and CV 1.60 – 14.0%.

In the reproducibility study data is evaluated, the recovery obtained for each residue at 10 ng g^{-1} of the matrix containing GC-MS/MS pesticides is 70.1% -120.0% and CV% 1.80 - 31.92%. The recovery achieved for 50 ng g^{-1} level is 70.7% - 120.7% and CV is 2.48 – 27.59%. It was found that the recovery obtained for each residue in the concentration range of 10-50 ng

g^{-1} of the matrix containing GC-MS / MS pesticides varied between 91.7-98.9% and CV 2.21-5.67%. Combining the recovery results from all the matrices and pesticides in GC-MS/MS resulted in 95.0% average recovery and 4.91% reproducibility % CVR of laboratory data.

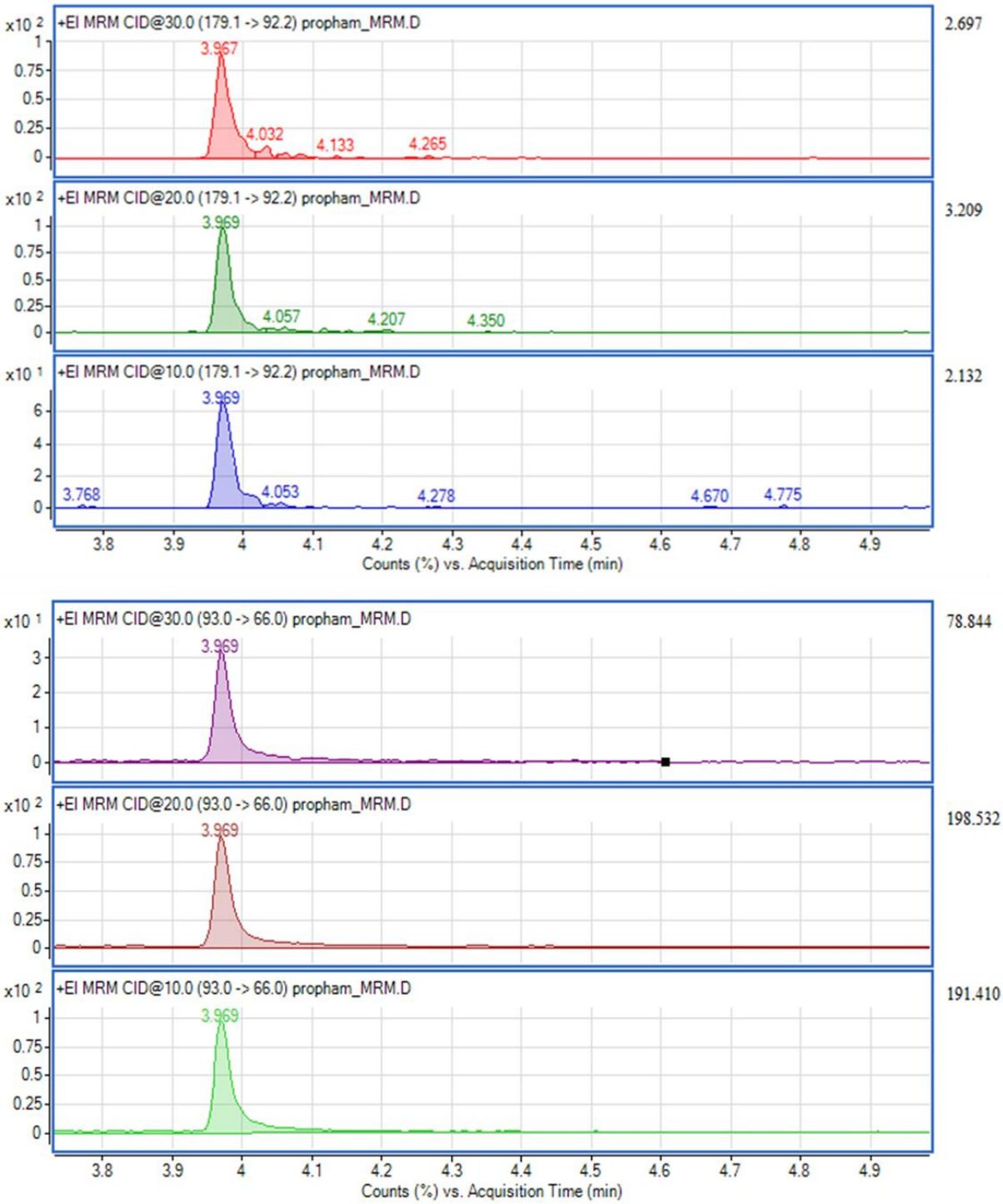

Figure 1. GC-MS/MS pesticide optimization evaluation for propham pesticide residue

4. CONCLUSION

This method is fast, both as an analysis method and as a method of reading on devices. The device can be analyzed in about 16 minutes in the European Union and in Russia with about 123 pesticides (with their metabolites) that are required for analysis.

Thus, approximately 123 pesticides were validated and quantified in GC-MS/MS. It covers a wide range of products from selected indications to vegetable origin food and food of animal origin. In addition, the method reduces time for analysis by providing time gain. On the other hand, the analysis cost has been reduced, and significant gains have been achieved on the basis of day-month-year as well as instrument consumables, analytical column, vial, working life of all working parts etc.

Acknowledgement

This study is part of the Ph.D thesis work carried out by the author Şeyda Kıvrak. It was financially supported by Scientific Research Project Office of Muğla Sıtkı Koçman University (Project No. BAP 12/06), held within Muğla Sıtkı Koçman University Science Faculty Chemistry Department as the doctorate study.

Conflict of Interest

The authors declare that there is no conflict of interests in this current study.

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