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RESEARCH ARTICLE

DETERMINATION OF POLAR PESTICIDES IN PLANT-BASED FOODS BY LC-MS/MS

Deniz ARSLAN BURAK [1,*], Yücel ŞAHİN [2], Muhammet BURAK [2]

[1] Occupational Health and Safety, Vocational School, Istanbul Arel University, Istanbul, Turkey.
[2] Department of Chemistry, Faculty of Science and Arts, Yildiz Technical University, Istanbul, Turkey.

ABSTRACT

Pesticides in plant-derived foods are becoming an important problem due to their intensive use in plant cultivation. Today, specific pesticides are used in agriculture. Ever since pesticides are potentially hazardous to the environment and thus to human health through the consumption of pesticide-contaminated food. The European Community, as well as the Ministry of Agriculture and Forestry of the Republic of Turkey, have set maximum residue levels (MRLs) for pesticide residues in plant-based foods, based on the assumption that good agricultural practices are applied in the use of pesticides in agriculture. As a result, food products must be checked to ensure that MRLs are not violated. Therefore, an appropriate control of its residues in the samples should be carried out.

In this study, polar pesticides in food based on plant origin were determined by LC-MS/MS after extraction with methanol according to modified Quick Polar Pesticides method which released and implemented by EURL. Optimization of 13 different polar pesticides was performed with LC-MS/MS Q-Trap. Once the optimization process was completed, the samples to be initialized according to the SANTE (Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed) guidelines have been carefully selected, including high water, high acid/high water, high sugar/low water, high oil/low water and high starch/low water content and difficult or unique commodities. Tomato, lemon, dried fig, red lentil, walnut and sage samples carefully selected according to SANTE guidelines, were run at two different concentration levels, at the detection limit of 10 µg/L and 100 µg/L. In the light of the studies, it was determined that the RSD (Relative Standard Deviation) criteria for reproducibility for all substances were below 20%. Furthermore, the recovery value for all substances was in the range of 70 – 120%. As a consequence, 13 different high-polar pesticide substances can be analyzed in plant-derived product groups with the LC-MS/MS method developed using a hypercarb column.

Keywords: Polar pesticide, SANTE, LC-MS/MS, MRL, QuPPe.

1. INTRODUCTION

Pesticides are chemicals used to kill pests that live or live on humans, animals and plants, and also reduce and damage their nutritional value during the production, preparation, storage and consumption of nutrients. These pests are parasites that carry various diseases, insects that are harmful to agriculture and plants, weeds and fungi, flying and walking creatures such as flies, lice, fleas, ticks, scabies, cockroaches in humans, animals, environment and shelters [1-3]. If pesticides are not applied, approximately 65% of product loss occurs [4].

Pesticides may leave a large amount of residue in foodstuffs in cases where pesticides are used above the recommended dose, applied more than necessary, mixed with more than one pesticide when necessary, or when the time required to be left between the last spraying and the harvest period is not respected.

Pesticides not only increase agricultural productivity, but also pose a serious threat to human and environmental health with the residues they leave when they are used unconsciously and incorrectly.

*Corresponding Author: denizarslanburak@arel.edu.tr
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Humans and other living things can be exposed to acute or chronic poisoning by consuming foods containing high doses of pesticide residues [5-11].

In addition to the obligation to use pesticides in agriculture, a tolerance value has been determined for pesticide residues due to poisoning and other toxic effects.

Pesticides in terms of food safety since 1962, European Food Safety Authority (EFSA) from 1976, Food Codex Commission (CAC) established under the World Health Organization (WHO), Food and Agriculture Organization (FAO) working under the United Nations at the international level. It evaluates the residues and determines the maximum residue limits that can be found in foods. In Turkey, pesticide residue limits are determined by the Ministry of Agriculture and Forestry. Applications are made according to the Turkish Food Codex Maximum Residue Limits Regulation of Pesticides, which was issued taking into account the European Union Directive No. 91/414/EEC and the relevant provisions of the European Union Parliament and Council Regulation No. 396/2005/EC.

Determination of high polarity pesticides by liquid chromatography mass spectrometry (LC-MS/MS) method is carried out successfully, especially in order to determine whether tolerance limits are exceeded in vegetable original foods [12-17].

The aim of this study is to analyze AMPA, glyphosate, glufosinate, ethephon, ethephon hydroxy, N-acetyl glufosinate, fosethyl aluminum, chlorate, perchlorate, MPPA, N-acetyl AMPA, maleic hydrazide and phosphonic acid to determine a new LC-MS/MS method for the qualitative and quantitative analysis of highly polar pesticides such as phosphonic acid to be performed in a short time, reliably and accurately.

The study consists of four stages. Determination of the most suitable conditions for the successful analysis of the pesticide components to be analyzed in the LC-MS/MS device in the first stage; In the second stage, pesticide components are extracted from plant-based food products using acidified methanol solution, in the third stage, standard solutions of different concentrations are prepared for the quantitative determination of each pesticide component, calibration curves are drawn in the LC-MS/MS device, and in the last stage, recovery studies are carried out using real samples and analysis is carried out. The performance of the method was measured.

LC-MS/MS device and extra optimization conditions were determined in order to perform the analysis method in the shortest time, with high efficiency and in the most accurate way.

2. MATERIALS AND METHODS

2.1. Chemicals

1 % (v/v) acidified methanol solution was prepared by adding 10 mL of formic acid to a 1000 mL flask and completing with methanol. A stock solution was prepared by dissolving the internal standard (Cyanuric acid) solution in water (LC-MS grade) at 1000 mg/kg. It was used in studies by diluting it with methanol as 10 mg/kg and 1 mg/kg. Calibration solutions were prepared as a mixture of 3 different concentrations, with the main stock standards being 10 mg/L. 10 mg/L intermediate stock mix solutions and 500 µg/L and 25 µg/L calibration mix solutions were prepared with LC-MS purity methanol.

2.2. Methods

To obtain a representative analytical portion from the laboratory sample, the sample is homogenized, from the homogenized sample to a 50 mL centrifuge tube with a cap, 10±0.1 g for wet products, 5±0.05 g for dry products (such as cereal, legumes, dried fruit, etc.) or 13.5±0.1 g (if the product is rehydrated

with water, that is, if 500 g of sample is added with 850 g of water and ground) and difficult products (such as spices, fruit tea, tea, etc.) are weighed 2.5±0.05 g.

Water was added to the tared centrifuge tube at the rate appropriate to the content of the product. Dry products were kept in water for 20 minutes for wetting after adding water. For 10 g sample, 100 µL of 10 mg/L cyanuric acid (internal standard) standard solution to 100 µg/L; 50 µL for 5 g sample; 25 µL was added for 2.5 g sample. 10 mL of acidified methanol was added to the prepared sample solution. The centrifuge tube should be shaken vigorously for 1 minute to effectively break up the crystalline aggregation. After the prepared solution was mixed in vortex, it was centrifuged at 4100 rpm at 10 ºC for 10 minutes. 3 mL of the centrifuged supernatant was taken and filtered through a 0.20 µ PET syringe filter into a 2 mL polypropylene vial. The filtered sample was diluted five times with water and analyzed by LC-MS/MS

2.2.1. LC-MS/MS Conditions

The mass spectrometry conditions for LC-MS/MS, AB Sciex 5500 Q-Trap, determined for the analysis method are given in Table 1.

Table 1. LC-MS/MS conditions

LC-MS/MS Parameters	Conditions
Ion Source (ESI, Turbo Ion Spray Mode)	Negative
Carrier Gas (Nitrogen) pressure	30psi
Collision Gas Level	Medium
Ion Spray Voltage	-4500V
Gas 1 (Zero Degree Air) Pressure	55psi
Gas 2 (Nitrogen) pressure	65psi
Gas 2 temperature	650 °C
Min/Max Waiting Time	80/300 minute

2.3. Validation Parameters and Criteria

For pesticide analysis, the SANTE [18] document titled “Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed.” numbered SANTE/11813/2017 is the main guide.

According to SANTE and UGRL [19] (National Food Reference Laboratory) guideline; linearity, sample effect (matrix effect), limit of detection (LOD) and limit of determination (LOQ), specificity, precision, robustness and bias are the parameters controlled during the validation of this study.

In the validation study, sample compatible calibrations were drawn at 7 different concentrations for 6 samples. Evaluation of sample-matched calibrations was done with a correlation value above a minimum of 0.95.

In this study, “matrix-matched calibration” was used to compensate for the matrix effect. Most remarkable way to eliminate the matrix effects of sample is to use matrix-matched calibration. Matrix-matched calibration works to compansate the effects of sample on calibration curve. To prepare the calibration curve level by level is way adding the standards as amounts by calculation of specified concentrations. Spiked samples for level of matrix-matched calibration curve is followed up in accordance with analysis procedure. Afterwards. each levels are injected to instrument to draw curve by concentration levels.

Within the scope of the analysis, 10 µg/L contamination studies were performed for each sample, with 10 replications in each product group, and the standard deviation of the results was taken.

In the SANTE document, specificity is defined as the detector's ability to provide the signal that will effectively identify the analyte (supported by selective extraction, cleaning, derivatization or separation where necessary). Retention times and signal heights in the LC-MS/MS device were considered distinctive for 13 different active substances. Retention times of high polarity pesticide active ingredients are given in Table 2.

Table 2. Retention times of pesticide agents

Pesticide	Retention time/min.
Ethephone	5.25
Glyphosate	2.34
Focetyl-Aluminium	4.52
Glufosinate	1.96
Maleic hydrazide	9.88
Chlorate	4.70
Perchlorate	9.47
N-Acetyl-AMPA	5.52
AMPA	1.14
HEPA	2.51
N-acetyl-Glufosinate	10.41
MPPA	5.55
Phosphonic acid	2.74

The SANTE document envisages the control of reality over the recovery values. Recovery values at both contamination concentrations should be in the range of 70-120%.

In the reality calculations, 10 and 100 µg/L contamination studies were carried out separately for tomato, lemon, dried fig, red lentil, walnut and sage samples, with a total of 10 repetitions, and the standard deviation of the results was taken. Accordingly, for the accuracy control, which constitutes a part of the accuracy parameter, 5 re-runs at 10 µg/L (at LOQ level) and 100 µg/L concentrations were performed to check the compliance of the recovery values to the range of 70-120%. The results were found to be appropriate.

Within the scope of this study, reproducibility and reproducibility studies were carried out. According to the SANTE document, the repeatability and reproducibility checks are made over the RSD values, and the compliance of the relevant RSD values with the ≤ 20% condition is checked. According to this for the repeatability control, which is one of the precision components, 5 re-runs were made at 10 µg/L (LOQ level) and 100 µg/L concentrations, and the conformity of the recovery values to the range of 70-120% was checked. The results were found to be appropriate and RSDr values of the measured values were calculated separately for each concentration and it was checked whether these values met the ≤20% condition.

According to the SANTE document, recovery studies should be carried out in "at least five repetitions" in contaminated samples at "at least two different concentrations" in method validation.

The first contamination concentration was chosen as a level below the maximum residue limit, and the second contamination concentration was chosen as a higher concentration.

According to the SANTE document, the reporting limit may be equal to or higher than the LOQ. Accordingly, in accordance with the SANTE document, validation studies were conducted in at least two concentrations, one of which must be at the LOQ level, and with at least five replications.

3. RESULTS and DISCUSSIONS

This analysis method, as indicated in Table 3, high water content, high acid and high water content, high sugar and low water content, high oil and very low water content, high oil and medium water content, high starch and/or protein content and low water and oil content products. The products include the analysis of 13 polar pesticides found in difficult or unique products. Pesticide agents: Ethephon, glyphosate, focetyl-aluminium, glufosinate, maleic hydrazide, chlorate, perchlorate, N-Acetyl-AMPA; AMPA, ethephon-hydroxy (HEPA), N-acetyl glufosinate, MPPA and phosphonic acid.

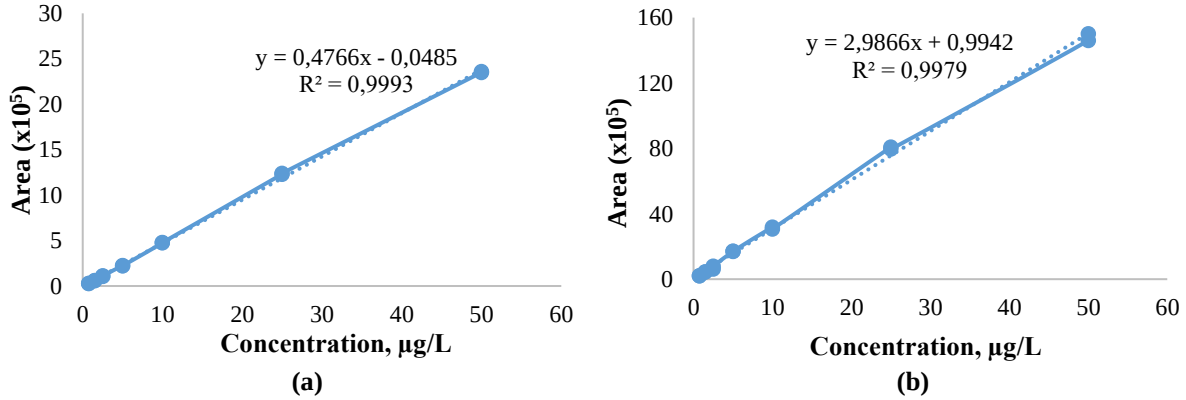
Table 3. Representative products used in the experimental study

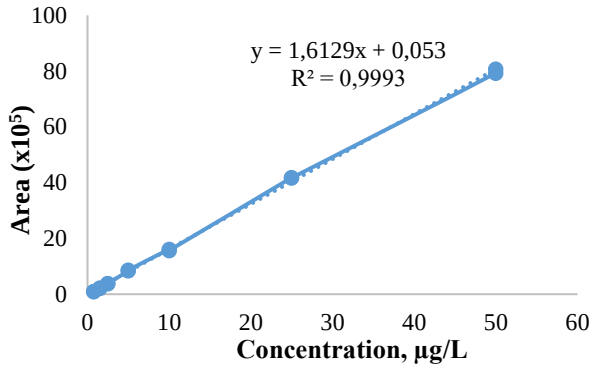
Product groups	Typical product categories	Selected representative products
Products with high water content	Seed vegetables / zucchini	Tomatoes
Products with high acid and high water content	Citrus	Lemon
Products with high sugar and low water content	Honey and dried fruits	Dry fig
Products with high oil and very low water content	Tree nuts	Walnut
Products with high starch and/or protein and low water and fat content	Dried beans/Legumes	Red lentil
Difficult or unique products		Sage tea

3.1. Verification

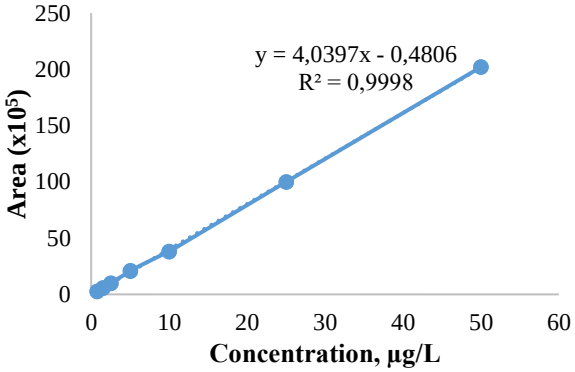
3.1.1. Linear calibration curve

As an example, as given in Figure 1 for the 13 polar pesticide agents, the linear measurement range, correlation values and sample-matched calibration curve studies performed with the blank sample showed the linearity, and the correlation curve value was obtained above the value of 0.95 as stated in the SANTE document. All relevant study results were evaluated according to these calibration curves.

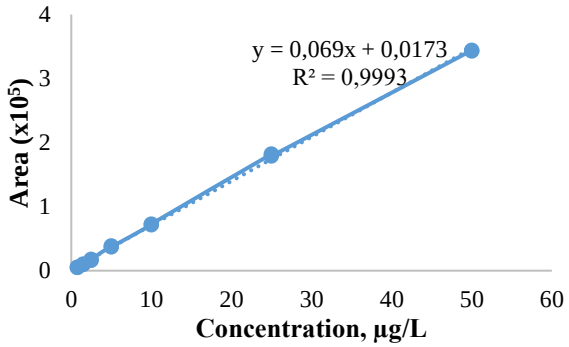



y = 1,6129x + 0,053
R² = 0,9993
Area (x10⁵)
Concentration, µg/L

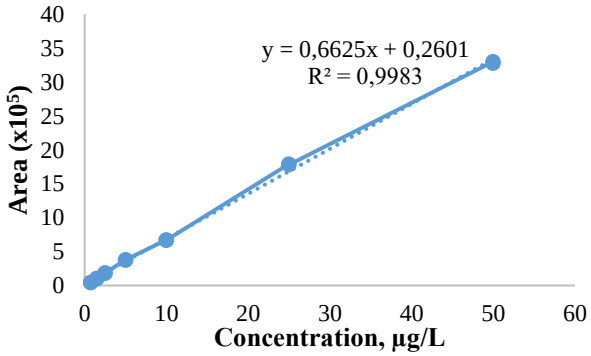
(c)


y = 4,0397x - 0,4806
R² = 0,9998
Area (x10⁵)
Concentration, µg/L

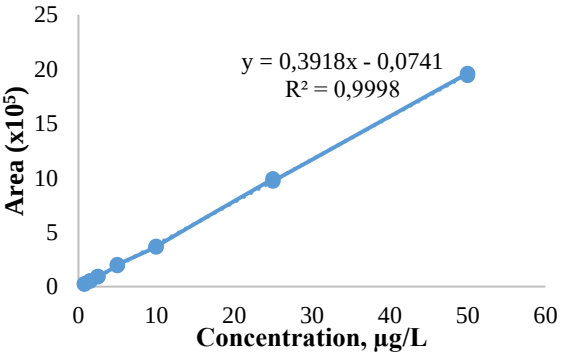
(d)


y = 0,069x + 0,0173
R² = 0,9993
Area (x10⁵)
Concentration, µg/L

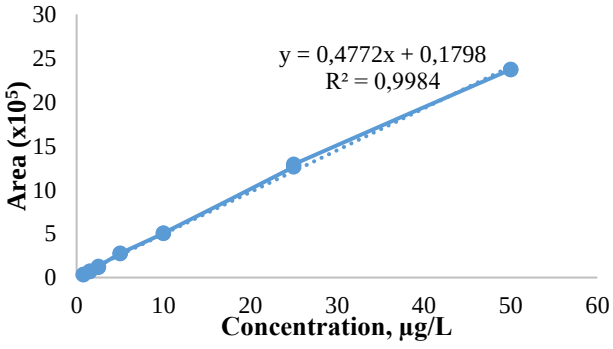
(e)


y = 0,6625x + 0,2601
R² = 0,9983
Area (x10⁵)
Concentration, µg/L

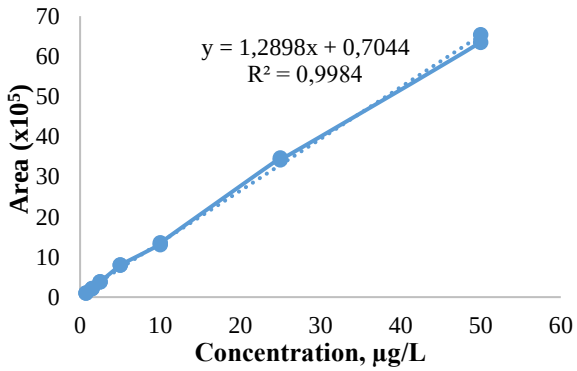
(f)


y = 0,3918x - 0,0741
R² = 0,9998
Area (x10⁵)
Concentration, µg/L

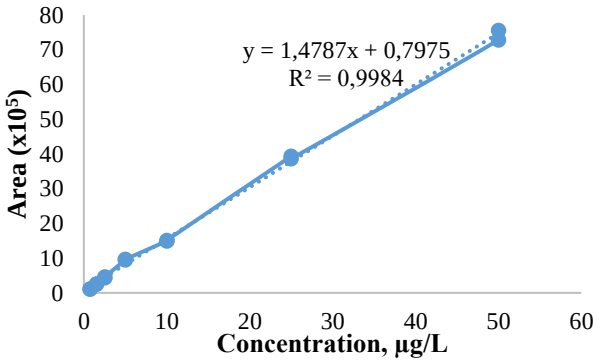
(g)


y = 0,4772x + 0,1798
R² = 0,9984
Area (x10⁵)
Concentration, µg/L

(h)


y = 1,2898x + 0,7044
R² = 0,9984
Area (x10⁵)
Concentration, µg/L

(i)


y = 1,4787x + 0,7975
R² = 0,9984
Area (x10⁵)
Concentration, µg/L

(j)

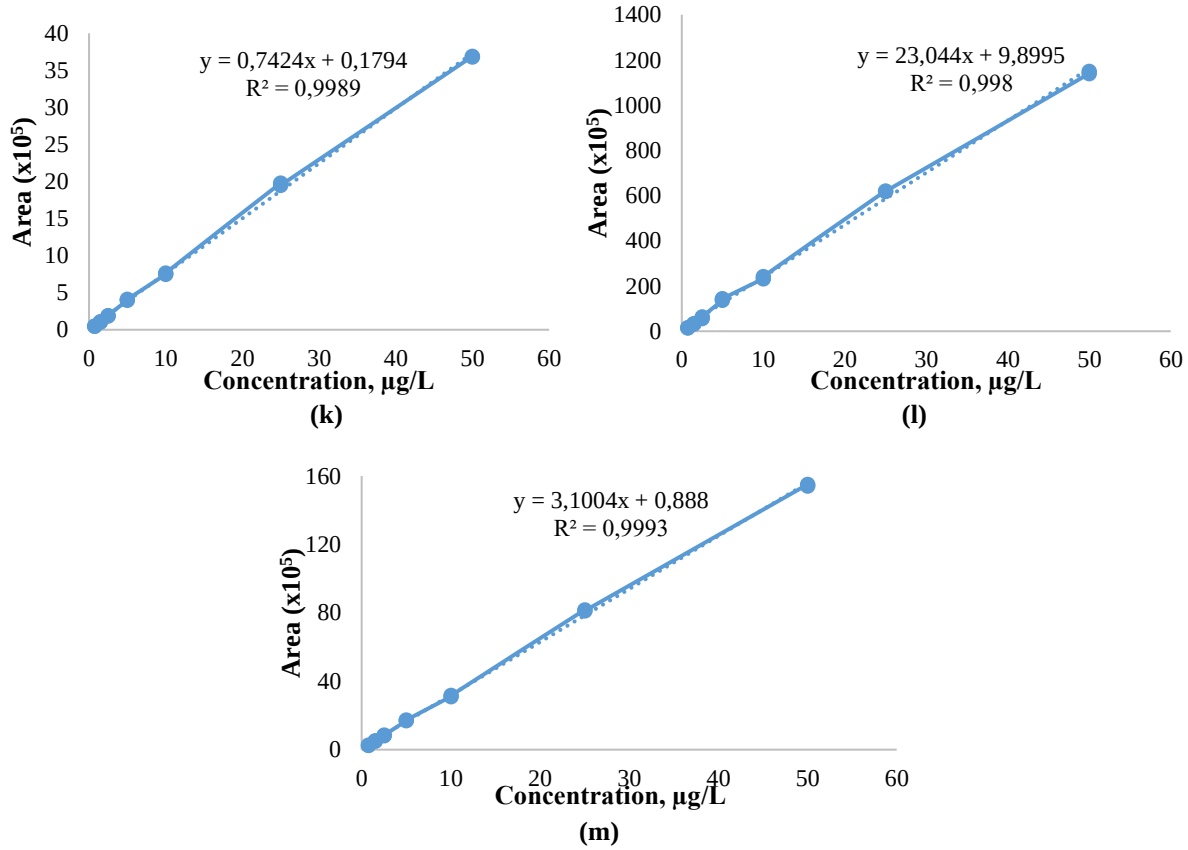


Figure 1. Calibration curve of (a) AMPA, (b) Chlorate, (c) Ethephone, (d) Focetyl-Aluminium, (e) Glufosinate, (f) Glyphosate, (g) HEPA, (h) Maleic hydrazide, (i) MPPA, (j) N-Acetyl-AMPA, (k) N-acetyl-Glufosinate, (l) Perchlorate, (m) Phosphonic acid

3.1.2. Limit of detection (LOD) and limit of quantification (LOQ)

Limit of detection (LOD) is the verified lowest residue concentration which can be quantified and reported by routine monitoring with verified methods. LOD is calculated as 3 times the standard deviation [18].

Limit of quantification (LOQ) is the lowest concentration or mass of the analyte that has been verified with approved accuracy by applying the complete analytical method. LOQ is calculated as 10 times the standard deviation [18].

Limit of detection and limit of determination values of 13 pesticide active ingredients in µg/L are given in Table 4.

3.1.3. Recovery studies

The recovery values calculated as a result of the studies carried out for reality were calculated separately for 13 different high polarity pesticides for each product group in studies belonging to two different levels. The recovery rates of the studies carried out are given in Table 5.

Table 4. LOD and LOQ values

Pesticides	Tomatoes		Lemon		Red lentil		Dry fig		Walnut		Sage tea	
	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
Ethephone	2.29	7.62	2.41	8.02	1.70	5.67	2.31	7.69	1.87	6.24	0.98	3.26
Glyphosate	1.31	4.35	1.43	4.78	0.56	1.86	2.81	9.38	1.52	5.06	2.35	7.84
Focetyl-Aluminium	1.13	3.76	1.77	5.90	1.22	4.05	1.06	3.52	1.39	4.65	1.32	4.40
Glufosinate	1.57	5.23	1.47	4.90	0.93	3.09	2.45	8.16	2.09	6.96	0.53	1.78
Maleic hydrazide	1.46	4.88	1.56	5.19	1.31	4.37	1.05	3.51	1.08	3.59	0.75	2.48
Chlorate	1.81	6.03	0.84	2.81	1.26	4.20	1.20	3.99	1.53	5.11	1.92	6.40
Perchlorate	2.65	8.85	1.51	5.04	1.43	4.78	1.41	4.69	0.68	2.27	0.91	3.03
N-Acetyl-AMPA	2.50	8.35	1.02	3.40	1.17	3.91	2.18	7.27	1.30	4.34	1.76	5.85
AMPA	1.35	4.50	1.76	5.88	1.40	4.65	1.59	5.32	1.38	4.61	0.79	2.62
HEPA	1.58	5.27	1.18	3.94	0.97	3.22	0.58	1.94	0.73	2.42	0.70	2.33
N-acetyl-Glufosinate	1.41	4.71	0.60	2.01	0.70	2.34	1.66	5.55	1.54	5.12	0.66	2.19
MPPA	2.86	9.52	0.81	2.71	0.83	2.76	2.68	8.92	2.77	9.23	2.99	9.95
Phosphonic acid	1.86	6.20	2.18	7.26	0.66	2.20	1.88	6.25	1.20	4.02	0.67	2.22

Table 5. Recovery rates of different products

Pesticide	Recovery, %					
	Tomatoes	Lemon	Red lentil	Dry fig	Walnut	Sage tea
Ethephone	87.0	92.0	91.9	89.3	87.4	83.1
Glyphosate	88.3	86.6	87.1	86.6	105.1	96.3
Focetyl-Aluminium	83.0	88.2	89.6	82.4	84.3	77.8
Glufosinate	83.1	82.7	95.7	85.2	87.8	79.3
Maleic hydrazide	82.8	85.1	87.1	79.9	81.8	77.1
Chlorate	89.0	97.9	96.2	84.9	108.7	101.5
Perchlorate	84.8	87.1	80.7	89.1	74.3	80.2
N-Acetyl-AMPA	88.6	86.4	84.7	94.2	91.9	86.5
AMPA	84.0	90.2	92.4	84.7	86.1	81.7
HEPA	81.9	83.3	85.4	78.8	84.6	77.6
N-acetyl-Glufosinate	89.6	85.0	102.2	92.7	99.0	93.0
MPPA	93.1	84.4	87.7	87.4	95.5	98.4
Phosphonic acid	86.3	85.8	93.6	79.4	79.6	78.5

3.1.4. Repeatability studies

The relative standard deviation (RSD) values of the studies performed meet the criteria of ≤20%, as stated in the SANTE document. The values of the studies carried out are given in Table 6 and Table 7.

Table 6. 10 µg/L RSD values

Pesticide	RSD,%					
	Tomatoes	Lemon	Red lentil	Dry fig	Walnut	Sage tea
Ethephone	8.79	7.96	5.50	7.76	7.23	3.85
Glyphosate	4.95	5.66	2.11	10.85	4.65	8.10
Focetyl-Aluminium	4.92	6.23	4.36	4.45	6.10	5.91
Glufosinate	6.16	5.63	2.64	8.55	8.14	2.40
Maleic hydrazide	5.97	5.92	4.79	4.54	4.65	3.38
Chlorate	7.21	2.46	3.62	5.17	4.46	6.00
Perchlorate	10.41	5.07	5.84	5.42	3.17	3.87
N-Acetyl-AMPA	10.46	4.60	5.25	8.43	5.84	7.01
AMPA	5.41	6.28	4.55	6.04	5.62	3.45
HEPA	6.75	4.76	3.88	2.67	3.27	3.19
N-acetyl-Glufosinate	5.80	2.81	2.03	6.31	6.14	2.47
MPPA	11.74	3.68	3.68	10.03	10.37	10.20
Phosphonic acid	7.35	8.98	1.91	7.92	5.25	2.98

Table 7. 100 µg/L RSD values

Pesticide	RSD,%					
	Tomatoes	Lemon	Red lentil	Dry fig	Walnut	Sage tea
Ethephone	4.06	5.31	6.44	4.27	5.09	4.68
Glyphosate	3.58	9.13	6.09	4.47	5.82	6.45
Focetyl-Aluminium	4.66	5.31	5.48	5.25	3.87	4.42
Glufosinate	3,78	5.86	5.36	3.48	2.31	4.92
Maleic hydrazide	4.40	5.22	5.43	3.87	2.70	4.41
Chlorate	3.72	5.88	4.45	5.15	4.01	8.84
Perchlorate	3.96	6.90	5.49	6.03	3.53	5.48
N-Acetyl-AMPA	3.77	7.46	12.69	5.76	2.36	5.30
AMPA	4.14	9.40	7.31	4.19	3.30	5.91
HEPA	2.37	4.19	6.37	4.32	2.35	4.21
N-acetyl-Glufosinate	2.89	10.57	8.02	4.83	3.02	4.54
MPPA	6.08	2.53	9.77	10.42	19.75	4.82
Phosphonic acid	3.46	3.51	4.49	4.44	4.57	3.19

3.1.5. Reproducibility studies

The RSD values of the studies carried out at low and high levels in six product groups on five different days meet the criteria of ≤20% as stated in the SANTE document. The values of the studies carried out are given in Table 8 and Table 9.

Table 8. 10 µg/L RSD values

Pesticide agent	RSD,%					
	Tomatoes	Lemon	Red lentil	Dry fig	Walnut	Sage tea
Ethephone	12.02	9.03	16.09	7.53	12.14	3.85
Glyphosate	10.69	7.28	18.82	9.52	20.35	8.10
Focetyl-Aluminium	15.58	10.63	11.71	17.22	16.19	5.91
Glufosinate	14.49	6.99	18.90	10.96	15.27	2.40
Maleic hydrazide	12.65	8.30	8.66	14.59	14.33	3.38
Chlorate	10.91	7.24	17.45	8.13	17.65	6.00
Perchlorate	8.97	7.41	6.04	6.27	4.99	3.87
N-Acetyl-AMPA	16.15	14.59	17.58	12.07	19.37	7.01
AMPA	11.38	6.15	12.03	7.24	12.48	3.45
HEPA	12.67	8.47	9.91	15.02	12.68	3.19
N-acetyl-Glufosinate	14.93	17.18	15.99	7.04	16.41	2.47
MPPA	17.21	6.60	19.91	10.79	12.03	10.20
Phosphonic acid	10.14	17.02	16.73	13.10	9.29	2.98

Table 9. 100 µg/L RSD values

Pesticide	RSD,%					
	Tomatoes	Lemon	Red lentil	Dry fig	Walnut	Sage tea
Ethephone	11.52	9.45	8.12	12.57	8.75	9,.0
Glyphosate	16.79	10.79	16.17	12.91	13.81	12.83
Focetyl-Aluminium	7.79	12.74	13.68	7.23	17.66	14.02
Glufosinate	15.45	18.49	9.73	16.04	8.94	17.61
Maleic hydrazide	12.70	7.92	11.40	9.55	14.23	10.21
Chlorate	13.89	11.28	15.52	15.91	11.34	12.79
Perchlorate	15.63	13.02	8.18	13.96	7.27	8.63
N-Acetyl-AMPA	14.99	10.87	13.24	15.26	18.07	9.39
AMPA	12.83	9.58	9.38	11.54	9.34	13.07
HEPA	14.84	16.77	8.85	17.10	10.66	18.60
N-acetyl-Glufosinate	9.23	4.28	8.05	6.47	10.46	6.88
MPPA	16.73	13.19	16.20	14.98	16.26	11.39
Phosphonic acid	15.83	19.94	10.22	15.68	13.76	16.26

3.2. Investigation of Updates to the Analysis Procedure

3.2.1. Comparison of injection volumes

As a result of the studies carried out by considering the original method source of QuPPe [20], injections at LOQ level were performed in 2 µL, 5 µL, 10 µL and 15 µL volumes to examine the effect of the injection amount on the analysis result. In order to examine the effect of injection amount on polar pesticide active substances, signal fields for each active substance are given in Table 9.

As shown in Figure 2 for the AMPA agent as an example, these injection volumes were not preferred due to the weak signals for 2 µL and 5 µL injection amounts. When the signals of 10 µL and 15 µL injection volumes were examined, it was observed that the peak shapes and areas of the peaks were higher. As a result of the recovery control of 10 µL and 15 µL volumes, it was determined that the values for all factors were more appropriate. In addition to all these examinations, a volume of 10 µL was accepted as the method injection amount in order to prevent contamination of the MS detector and LC system.

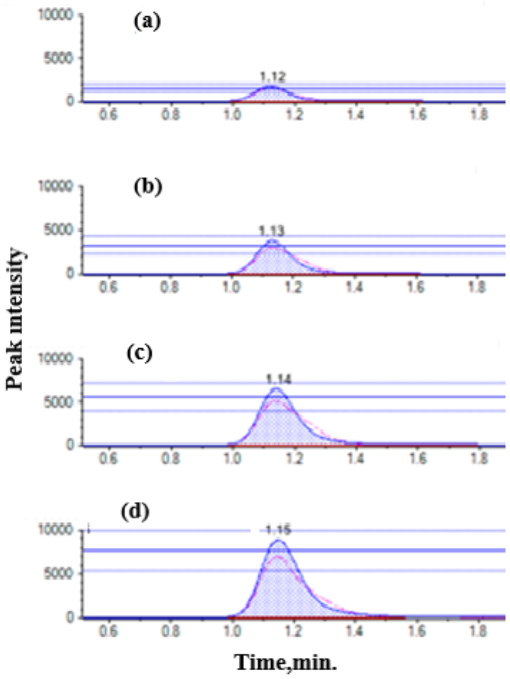


Figure 2. AMPA agent obtained in different injections (a) 2µL (b) 5µL (c) 10µL (d) 15µL

3.2.2. Comparison of dilution coefficients

After the extraction phase was completed, the peak shapes formed between the injection of the sample directly into the device after filtration and the injection as a result of different dilutions were examined. Based on the QuPPe method, it is recommended that the sample be injected directly into the device after extraction. As a result of the studies, it has been observed that the part of the extracted sample is directly injected into the device, resulting in distortion in the peak shapes and signals are obtained in a sparse manner. At the same time, it was observed that the signal heights obtained as a result of direct injection were lower than the signals obtained by dilution. In order to examine the dilution step after extraction, the sample was diluted 2, 5 and 10 times with ultrapure water in the last step.

As a result of multiple replication studies, it was seen that the signals of the samples diluted 10 times were sharper and the peak heights were better than the other diluted samples, as in the AMPA factor in Figure 3. At the same time, dilution prevents contamination that may occur in the device. As a result of these studies, the method dilution coefficient was accepted as 10.

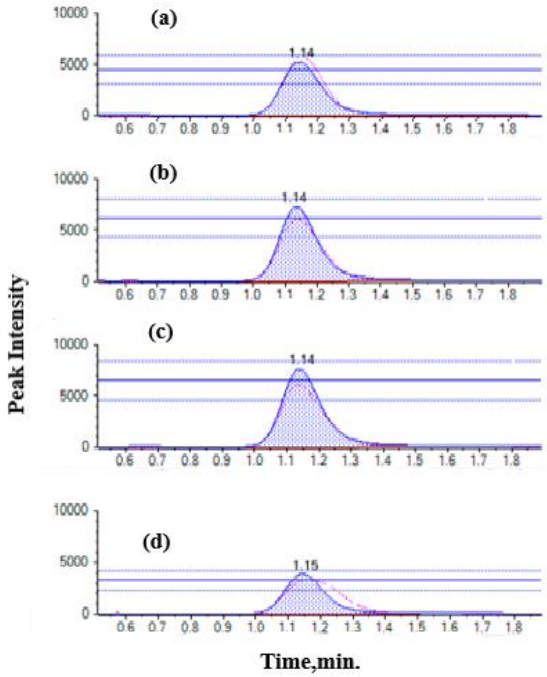


Figure 3. AMPA agent obtained at different dilution coefficients (a) 2 times dilution (b) 5 times dilution (c) 10 times dilution (d) Direct injection

3.2.3. Comparison of run time

There are two screening and quantitative methods published in QuPPe method version 10 and defined for 13 active substances. In the first of these methods, the ethephone; glyphosate; focetyl-aluminum; glufosinate; maleic hydrazide; N-Acetyl-AMPA; AMPA; HEPA; N-acetyl-glufosinate and MPPA agents; chlorate in the other; It was aimed to analyze perchlorate and phosphonic acid agents. The total time required for two different injections of these methods for a sample analysis is 45 minutes. In our study, as a result of combining two different methods, it was aimed to analyze 13 active substances in one study period and in the shortest time. By keeping the chromatographic column and mobile phases used in the relevant analysis method constant, the pump flow and the UPLC flow program were changed, making it possible to analyze all factors in a shorter time.

As a result of the injections, the method run time was carried out in as little as 20 minutes, and at the same time, 13 active substances were detected and determined together. For the AMPA agent, the signals of the peaks obtained as a result of 20 minutes (a) and 30 minutes (b) run times are given in Figure 4. As a result of the studies, the comparisons of the retention times of the QuPPe method flow program and the thesis study flow programs are given in Table 10.

Furthermore**,** the results in Table 11 were performed according to the technical data sheet of the analysis for each blank matrix. Verification of the analysis was performed with blank samples deemed 'Not Detected' for each component.

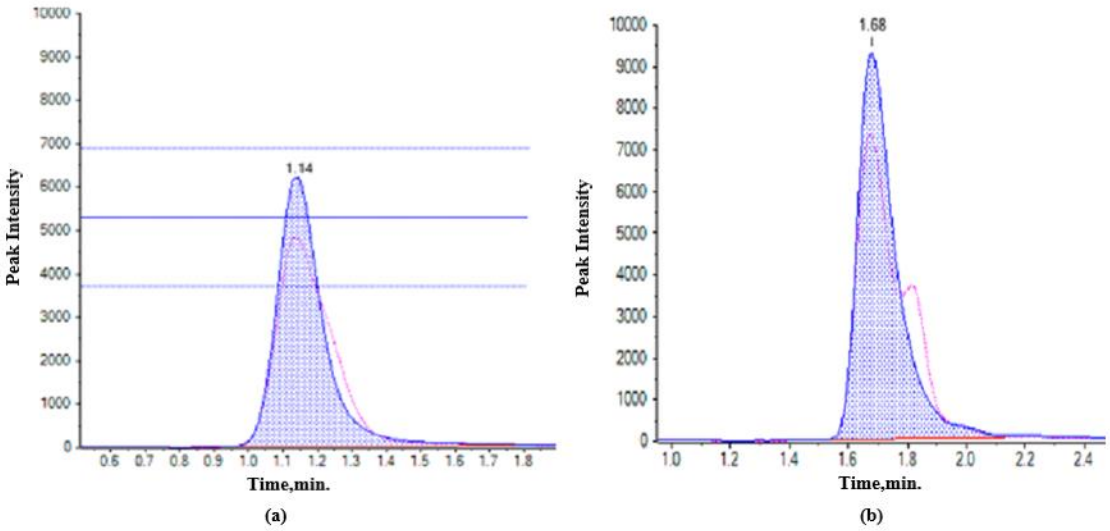


Figure 4. Signals and retention times related to the run time of AMPA agent (a) 20 minutes (b) 30 minutes

Table 10. This method and QuPPe method flow programs retention times

Pesticide	Retention time/min.	
	Improved Method	QuPPe Method
Ethephone	1.14	1.68
Glyphosate	4.72	7.16
Focetyl-Aluminium	5.33	8.26
Glufosinate	2.54	3.81
Maleic hydrazide	4.61	7.04
Chlorate	1.98	2.97
Perchlorate	2.36	3.54
N-Acetyl-AMPA	9.82	11.43
AMPA	5.60	8.02
HEPA	5.57	8.11
N-acetyl-Glufosinate	10.36	11.96
MPPA	9.37	12.74
Phosphonic acid	2.74	4.11

Table 11. Results of blank samples for each substance

Pesticide	Blank Results, µg/L					
	Tomatoes	Lemon	Red lentil	Dry fig	Walnut	Sage tea
Ethephone	0.52	0.15	0.12	0.27	0.75	0.60
Glyphosate	0.49	0.19	0.17	0.21	0.81	0.63
Focetyl-Aluminium	0.50	0.14	0.18	0.23	0.76	0.62
Glufosinate	0.45	0.19	0.13	0.24	0.74	0.61
Maleic hydrazide	0.51	0.12	0.10	0.25	0.73	0.61
Chlorate	0.43	0.18	0.12	0.31	0.74	0.69
Perchlorate	0.43	0.12	0.18	0.26	0.67	0.63
N-Acetyl-AMPA	0.49	0.17	0.14	0.26	0.77	0.69
AMPA	0.41	0.18	0.18	0.24	0.74	0.67
HEPA	0.55	0.17	0.15	0.20	0.66	0.60
N-acetyl-Glufosinate	0.43	0.18	0.15	0.27	0.76	0.68
MPPA	0.53	0.19	0.10	0.28	0.76	0.69
Phosphonic acid	0.50	0.14	0.12	0.28	0.76	0.66

4. CONCLUSION

As a result, an analytical method has been developed that enables the simultaneous quantitative determination of 13 different pesticide active ingredients. With the help of this method, it is possible to analyze pesticides with different high polarity, which cannot be analyzed together at the same time. According to the QuPPe method, 13 pesticides, which were analyzed with two different analytical methods, were analyzed with a single method.

The most suitable conditions were determined by analyzing 13 pesticide active ingredients in different injection volumes and using different dilution factors, and the precision of the analytical method was increased by enabling analysis in a short time like 20 minutes.

Reproducibility and reproducibility studies were carried out with the analytical method determined for each pesticide agent, and some statistical calculations were made as a result of these studies. As a result of these calculations, the relative standard deviation value for each active substance of the analysis method was below the 20% criterion and the recovery value was between 70-120%. At the same time, the expanded resultant uncertainty value calculated as a result of the repeatability and reproducibility studies was obtained below the 50% criterion as expected for each pesticide agent.

In this study, 13 different polar pesticides were determined simultaneously by the developed QuPPe method. Compared to similar studies in the literature, method superiority in LOQ, RSD and Recovery values according to the commodity of samples was compared and this study was found to be more advantageous. In a study [12] in which the active ingredient glufosinate was performed using the QuPPe method, it was observed that the RSD values obtained at 10 and 100 µg/L levels were higher than the RSD values obtained in this study. In the literature, in a different combined analysis method [13], it was observed that the values obtained as a result of LOQ studies carried out in the oily product group were higher than the values obtained in this study in similar product group as well. In a study where the QuPPe method was not used in the literature, but the parameters of maleic hydrazide, glyphosate, fosetyl-Al, and ethephon were performed, which was carried out as a similar rapid method [14], it was understood that the LOQ and recovery values obtained in this study were more advantageous compared to the values obtained in a similar product group.

As a continuation of the study, it is aimed to analyze more pesticide agents with the same method by increasing the number of pesticide active ingredients with high polarity.

CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article.

AUTHORSHIP CONTRIBUTIONS

The authors contributed equally to this work.

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