Journal of Al Azhar University-Gaza (Medical and Health Sciences), 2022, p: 14-29

Determination of Chlorpyrifos Residues in Tomato Marketed in the Gaza Strip Using QuEChERs Extraction and HPLC-Fluorescence Detection

Mai Ramadan* , Sahar El-Najjar

Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmacy, Al-Azhar University-Gaza, Palestine.

(Received 10/08/2022, Accepted 16/11/2022)

تحليل متبقيات الكلوربيرفوس في الطماطم المتداولة في قطاع غزة عبر تقنية الاستخلاص QuChERs، والتحليل الكروماتوجرافي السائل عالي الأداء المقترن بالكاشف الفلوري

مي رمضان & سهر النجار

[*1] قسم الكيمياء الصيدلانية والعقاقير، جامعة الأزهر-غزة، فلسطين.

(تاريخ الاستلام 2022/08/10، تاريخ القبول 2022/11/16)

[*] المؤلف المراسل: مي رمضان، قسم الكيمياء الصيدلانية والعقاقير، جامعة الأزهر-غزة ، فلسطين

[*] **Contact**:
Mai Ramadan, Department of Pharmaceutical Chemistry and Pharmacognosy, Al-Azhar University, Gaza Strip, Palestine
Email: m.ramadan@alazhar.edu.ps

Abstract

This study presents the application and testing the efficacy of QuChERs for extraction of chlorpyrifos (CF) from tomato. Extraction procedure follows AOAC 2007.01 official method. For analysis a HPLC coupled with fluorescence detector was verified according to SANTE/2020 guidance on pesticide analytical methods in terms of linearity, range, recovery, and precision.

The chromatographic conditions were C18-column as stationary phase, and a mixture of methanol: water (95:5 v/v) as mobile phase in isocratic elution pattern and a flow rate of 0.8 mL/min. The fluorescence detection parameters were λ excitation: 280 nm, and λ emission: 340 nm.

A matrix matched calibration curve was Y= (724169+2.3%) X + (459043+10.8%), R2 = 0.996+0.26% (n =3), where Y: Peak area, and X: Concentration (µg/g). Range was 0.05 -10.0 (µg/g). Percent recovery ranged from 77.5 to 109.4%, and percent relative standard deviation for intra- and inter-day precision was less than 20%. Hence, it was suitable for determination of CF in tomato.

CF was detected in three tomato samples (Total samples: 7) at a concentration of 1.25 to 5.51 (µg/g), which was above the maximum residue limit (MRL). Samples were collected from different local markets. Routine analysis of pesticide in vegetables and equipping quality control laboratories in Gaza Strip is advised .

Keywords: Chlorpyrifos, QuChERs, HPLC, Fluorescence, MRL.

الملخص:

تتناول الدراسه فحص الكفاءه لتقنية الاستخلاص QuChERs في استخلاص الكلوربيروفوس من الطماطم والتي تتبع طريقه2007.01 (AOAC) وعملية تحليل المستخلص تمت بتقنية الكروماتوجرافي السائل عالي الأداء (HPLC) المقترن بالكاشف الفلوري والتي خضعت لعملية فحص الكفاءه تبعا لقواعد SANTE/2020 الخاصه بتحليل المبيدات من حيث المعادلة الخطية ومجالها، كفاءة الاستخلاص، والدقة.

الفصل الكروماتوجرافي اعتمد على استخدام العمود الكروماتوجرافي Shim Pack-C18 والخليط من الميثانول والماء بنسبة (5:95) كطور متحرك بشكل مباشر وبسرعة 0,8 ميليلتر/دقيقة. أما الكاشف الفلوري تم ضبط الطول الموجي للاثارة (λEx) على 280 نانومتر والطول الموجي للانبعاث (λEm) علي340 نانومتر.

المعادلة الخطية هي: ص= (724169 ± 2,3%) س+ (459043 ± 10,8%) ومعامل الارتباط 0,996 ± 0,26% للعدد 3 اعتمادا علي المستخلصات بعد المزج علما بأن ص: مساحة الذروة ، س: تركيز بوحدة ميكروجرام لكل جرام في المجال من 0,05 الى 10 ميكروجرام لكل جرام. نتائج دراسة كفاءة الاستخلاص تراوحت بين 77,5% و 109,4%. أما فحص الدقة في اليوم الواحد وعلى عدة أيام سجلت له نسبة الانحراف المعياري (RSD%) بأقل من 20%. وبناءا على هذه النتائج تم استخدام الطريقة في تحليل العينات.

تم الكشف عن الكلوربيرفوس في تلات عينات من الطماطم (العدد الكلي =7) والذي بلغ بين 51,2 و 15,5 ميكروجرام لكل جرام وهو أعلى من الحد الأقصى المسموح به. هذه العينات تم جمعها من الأسواق العامة. هذه النتائج تدل على ضرورة اجراء التحاليل الدورية لمتبقيات المبيدات في الخضار المتداولة في قطاع غزة إضافة إلى تجهيز المختبرات الرقابية للجودة.

الكلمات المفتاحيه: كلوربيرفوس، QuChERs، التحليل الكروماتوجرافي السائل عالي الأداء، الفلوري، الحد الأقصى للمواد المتبقية.

Introduction

Pesticides are widely utilized in cultivation of agricultural crops, which are vulnerable to attacks by insects and diseases. Although the use of pesticides provides unquestionable benefits in terms of delivering a plentiful, low cost supply of high quality fruits and vegetables, their improper application can result in toxic residues that pose health risk (Gupta, 2011). Therefore, there is increasing interest in identifying and quantifying pesticide residues in agricultural food consumed by human (Hercegová *et al*., 2007; Yuan *et al*., 2014, Akhtar et al., 2009). Pesticide maximum residue limits (MRLs) in food commodities were regulated by Codex Alimentarius in response to growing public concern over the potential health risk associated with exposure to pesticides (Codex Alimentarius Commission, 2007, Casida and Bryant, 2017). Tomatoes (*Solanum lycopersicum* L.) are the world's second-most important vegetable after potatoes (Dorais *et al.*, 2008). Gaza Strip annually produces about 80 thousand tons of tomatoes, of which about 20 thousand tons are exported to the West Bank and some Arab countries. The rest is consumed inside the Strip with 200 tons sold daily (PCBS, 2010).

Reports showed that about 900 metric tons of formulated pesticides are used in the Gaza Strip annually mainly of organochlorinated and organophosphate type. A progressive increase in application of pesticides in agricultural sector Gaza Strip-Palestine was reported in the last two decades (Safi *et al.*, 2002, Batta, 2002, Al-Sa'ed *et al.*, 2011, Safi *et al.*, 2001, Al-Kurdi *et al.*, 2018, El-Nahal, 2004, El-Nahal and Safi, 2012, ARIJ, 2020).

Chlorpyrifos (Trade name Dursban) is an organophosphorothionate insecticide with the chemical name O, O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothionate. Figure 1 shows the structure of chlorpyrifos (CF). It is used primarily to control foliage and soil-borne insect pests (PPDB, 2020, Lewis et al., 2017, Medina-Pastor and Triacchini, 2020).

Although CF is banned in Europe and the MRL was established at 0.01 ppm in June 2020, it is still applied widely in the Gaza Strip for tomatoes and other crops (Codex Alimentarius Commission, 2020, PPDB, 2020).

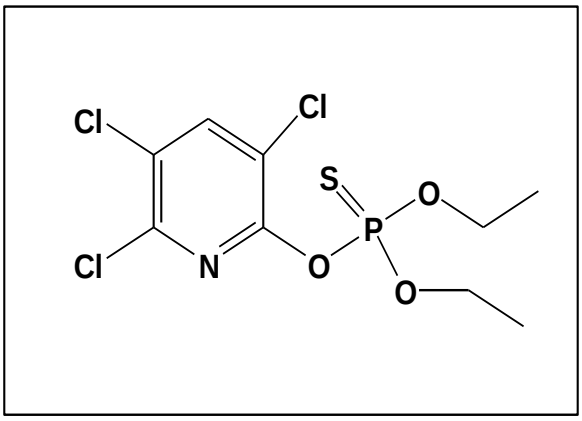

Figure 1: Chemical structure of Chlorpyrifos.

Pesticide analysis methodologies usually in ultra-traces range (ppm) require typically separative analytical techniques such as gas chromatography GC and liquid chromatography LC. The most widely used technique is GC in multi-pesticide residue

analysis due to high resolution capacity and the availability of selective detectors such as electron capture, flame photometric, nitrogen phosphorus, and mass spectrometric detection (Tankiewicz, 2019, Narenderan *et al.*, 2020, Mohammed *et al.*, 2019). Methods based on LC have been used rarely with detectors namely UV, DAD and fluorescence due to its low sensitivity and selectivity (Wahab *et al.*, 2022, Hazar *et al.*, 2017, Ramadan *et al.*, 2016). LC with mass spectrometric detection is used to quantify very polar insecticide residues (Stachuink *et al.*, 2017, Song *et al*., 2020, Lawal *et al.*, 2018).

To date, several techniques have been developed for the extraction of pesticide from different matrices. It ranges from traditional techniques e. g. liquid-liquid -, sohxlet-, supercritical fluid -, pressurized liquid-, and microwave assisted extraction to advanced techniques like solid phase extraction, matrix solid phase dispersion, molecular imprinted polymers, and QuChERs (Samsidar *et al.*, 2017, Narenderan *et al.*, 2020).

QuChERs is a technology that greatly simplifies the analysis of pesticide residues. It's quick, easy, cheap, effective, rugged, and safe. It consists of two primary steps: extraction of analytes of interest with extraction salts, and cleaning up of extract with dSPE (Capriotti *et al.*, 2013, Kim *et al.*, 2019, Rejczak and Tuzimski, 2015, Perestrelo *et al.*, 2019).

Few studies were performed to analyze pesticide residues in vegetables cultivated and marketed in Palestinian territories (El Najar, 2001, Yahya, 2021, Safi *et al.*, 2002, Safi *et al.*, 2001, Al-Kurdi *et al.*, 2018). This study aimed to apply the Association of Official Agricultural Chemists (AOAC) 2007.01 official method for effective extraction and to verify a HPLC coupled with fluorescence detection method (Hazar *et al.*, 2017) according to SANTE/2020 guidelines in our laboratories (European Commission, 2021). After effective completeness of the examinations, the procedure was applied to real samples. Residual level of CF was monitored in tomato as a non-seasonal vegetable – cultivated and marketed in the Gaza Strip.

Materials and methods

Instruments

HPLC apparatus was Shimadzu HPLC prominence LC–20AT solvent delivery pump equipped with vacuum degasser (DUG–20A5R), combined with system controller (CBM–20A Lite) and fluorescence detector (RF–20A), and LC solution software. It is equipped with 20 µl injection stainless steel loop (shimadzu, Japan).Shim-pack® VP-ODS column (250 × 4.6 mm, 5 µm) (shimadzu, Japan), Shim-pack® VP-ODS Guard column, (GSIL 7333) (shimadzu, Japan).

Other instruments used in the procedure were digital Balance (Boeco, Germany), vortex (IKA®/ Germany), centrifuge (Centric 400R, Domel, Slovenia), refrigerator (Munro, UK), rotatory evaporator (RE100-Pro., Scilogex, USA).

Chemicals and materials

HPLC-grade solvents e.g. acetonitrile, methanol and water were purchased from Sigma-Aldrich, Germany. Chemicals e.g. chlorpyrifos standard (Sigma-Aldrich, Germany), MgSO4, NaCl, sodium acetate, acetic acid p.a. grade (Sigma-Aldrich, Germany) were obtained from drug and toxicology analysis centre, Al-Azhar University-Gaza.

Restek Q-Sep QuEChERs dSPE tubes for extract clean up (Restek cat no. 26224] – contain 900 mg MgSO4, 150 mg PSA, 15 mg GCB in 15 ml centrifuge tube - were purchased from Restek, Germany.

Other materials syringe nylon filters 0.2 µm, centrifuge tubes of 50 mL, and 15 mL (Macherey-Nagel, Germany) were used.

Reagents

CF standard stock solution was prepared by dissolving 5 mg standard in 5 mL volumetric flask using acetonitrile HPLC (1,000 µg/mL).

CF working standard solutions were prepared by diluting appropriate volumes of standard stock solution with acetonitrile to produce (10 µg/mL). The solutions were kept in refrigerator at -20°C, which are stable up to six months (Hazer et al., 2017).

Sampling and subsampling

One kilogram of tomato was collected randomly from each shop (4 shops) per market within the main vegetables market of 6 governorates in the Gaza Strip [Rafah, Khanyounis, Deir El Balah, Gaza, Beit Lahia, and Beit Hanoun] in October, 2021. Tomato collected per market were mixed and one kilogram (ca. 12 items) of the mixture is considered a representative sample of the market. The representative samples were transported to the laboratory in clean plastic packages. Sample were cut, crushed, and preserved in plastic containers. Each package was given a number according to each region and stored in the refrigerator at -20 °C (Commission of the European Communities, 2002, FAO, 1988).

Blank tomato (Designated to be organic product and tested in Lab) was collected from a farm in Deir El Balah.

Chromatographic conditions

The chromatographic conditions were a mixture of methanol: water; 95: 5 (v/v) as a mobile phase in isocratic elution pattern within a flow rate of 0.8 mL/min, at room temperature, Shim-pack® VP-ODS column (250 × 4.6 mm, 5 µm) as a stationary phase, and fluorescence detector adjusted at λ excitation: 280 nm, and λ emission: 340 nm. The injection volume was 20 µL (Hazer et al., 2017).

Preparation of spiked samples

To ten grams of blank tomato homogenate in a 50 mL centrifuge tube the following volumes 10, 50, 80, and 100 µL of (CF 1,000 µg/mL standard) were transferred, vortexed to produce 1, 5, 8, and 10 (µg/g).

To prepare 0.05, 0.1, and 0.5 (µg/g) spiked samples 50, 100, and 500 µL of CF standard (10 µg/ml) were mixed with 10 g blank tomato.

Extraction of samples

Tomato homogenate was thawed and allowed to reach RT. Ten grams of tomato homogenate were weighed in 50 mL centrifuge tube. 10 ml of acetonitrile containing acetic acid 1% were added, and vortex for 30 seconds. A mixture of 5.0 g MgSO4, 1.0 g Na acetate, and 1.0 g NaCl was added and shaken vigorously for 3 minutes, then centrifuge at 4000 rpm for 10 minutes. 7.0 mL of the supernatant were transferred into the dSPE clean-up centrifuge tube [A mixture of 900 mg MgSO4, 150 mg PSA (Primary Secondary Amine), 15 mg GCB (Graphitized Carbon Black)], shaken well, then centrifuged at 4000 rpm for 10 minutes. The supernatant was put in a clean round Kolbe, and evaporized at a temperature not exceeded 40 °C using the rotatory evaporator. The residue was kept at -20 °C until being analysed. For analysis the residue was dissolved in 1.0 mL acetonitrile, shaken well, and filtered using a syringe nylon filters 0.2 µm.

Matrix effect

Ten grams of blank tomato were extracted and cleaned up as described above. Blank extract was used as diluent to prepare a CF-standard at a concentration of 5.0 (µg/mL).

Matrix effect (ME) was calculated using equation:

$$\%\mathbf{ME} = \frac{\mathbf{X2} - \mathbf{X1}}{\mathbf{X1}} \times \mathbf{100}$$

X1 is the average area of CF 5 (µg/mL) diluted in solvent, and X2 is the average area of CF 5 (µg/mL) diluted in blank extract.

Method verification

Hazer et al. method was applied in the laboratory. Application of analytical method for traces analysis required a verification (European Commission, 2021). The verification included the following parameters selectivity, linearity and range, accuracy, precision, LOD and LOQ.

Selectivity

Selectivity of an analytical method is its ability to measure accurately an analyte in the presence of interferences that may be expected to be present in the sample matrix. For this purpose the chromatogram of CF standard, blank, and spiked sample were recorded.

Linearity and range

A matrix matched calibration curve was used in this method. Spiked samples (0.05-10.0 µg/g) were extracted as described above. The area of the peak was plotted against the concentration. The calibration curve was calculated using Microsoft Excel program. The procedure was repeated 3 times within a weak and the average equation was used for estimation of CF quantitatively.

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the matrix (Including these concentrations) for which it has been demonstrated that the analytical

procedure has a suitable level of precision, accuracy and linearity.

Accuracy

Accuracy of the method was ascertained by performing recovery studies. Spiked samples at three different levels 0.05, 1.0, and 5.0 (µg/g) were analyzed and the concentration was calculated using the matrix matched calibration curve. The percent recovery was calculated using the following formula

$$\% \textbf{ Recovery} = \frac{\textbf{Conc. calc}}{\textbf{Conc theor.}} \times \textbf{100}$$

Conc. calc. is the concentration calculated by the matrix matched curve, and Conc. theor. is the theoretical concentration.

Precision

Intra-day precision was assessed by analysis of spiked samples at three different concentrations [0.05, 1.0, and 5.0 (µg/g)] at three different time periods of the same day. The relative standard deviation (RSD) was calculated for each concentration. On the other hand, inter-day precision was studied by repeating the procedure for three different concentrations (0.05, 1.0, and 5.0 (µg/g)] within a week and % RSD was calculated for each concentration.

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using formulas

$$\textbf{LOD} = \textbf{3.3}\ \frac{\sigma}{\textbf{S}} \text{ and } \textbf{LOQ} = \textbf{10}\ \frac{\sigma}{\textbf{S}}$$

σ is the standard deviation of residuals of the regression line, and S is the slope of the calibration curve.

Analysis of tomato samples

The sample homogenate were extracted and analyzed as described above. The area of the peak at the retention time of CF was recorded. The concentration was calculated using the matrix matched calibration curve.

Results and discussion

Tomato (*Lycopersicon esculentum*) is the second-most important vegetable in the world after potato (Dorais *et al*., 2008). It is cultivated in the Gaza Strip and is mainly consumed locally (PCBS, 2010). This study aimed to investigate the residual pesticide namely CF in tomato to apply trace analysis in our laboratory using modern QuChERs protocol for extraction and find out the analytical obstacles. In addition, to examine the pesticide residue as a contributor of health problems in such a non-seasonal important vegetable (ARIJ, 2020).

Extraction procedure was applied successfully (Figure 2). The first step of extraction produced three layers and the supernatant was further cleaned up to produce the mostly decolorized extract. Ceramic homogenizer was not required to enhance the first extraction step.

Matrix effect

Matrix effect (ME) is the assessment of the effect, if any, of co-extractives on the analyte response. It is obtained by comparing standard prepared in extract of representative commodities (Tomato) with those in solvent. ME examined at a CF-concentration of (5 µg/mL) was 21.97%. This showed elevated response of detectors by matrix co-extractives in this case.

Verification of the method

The method of Hazer et al. for trace analysis of CF was applied in laboratories of Al-Azhar University–Gaza. The method was examined before application according to the European commission guidelines. For this purpose, the following parameters were investigated.

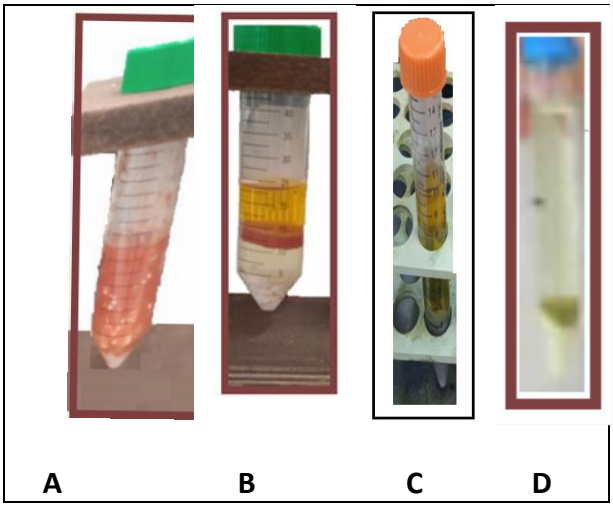

Figure 2: QuChERs extraction process. A: Tomato mixed with extraction salts, B: Extract after centrifugation, C: Tomato extract mixed with clean up salts, and D: Extract after clean-up step and centrifugation.

Selectivity

Chromatograms in figure 3 show the peak of CF detected by fluorescence detector at a retention time of 9.6 minutes. No interferences were detected at this time. The blank chromatogram showed no peaks which indicated good agreement with organic product characterization. Chromatogram of CF standard working solution showed many peaks eluted before 5.5 minutes. The source of co-eluted substances was explained when acetonitrile HPLC grade was injected onto the column. This indicated uncertainty of HPLC grade solvents available on the market in the Gaza Strip. This problem is serious in trace analysis. In this case it did not disturb the process.

Linearity and range

The calibration curve was constructed by plotting the average area of CF as a function of the corresponding concentration in spiked samples (µg/g). The linearity data are given in table 1. A linear relationship was achieved in the range of 0.05 to 10.0 (µg/g) within a regression factor of R^2= 0.9964 ± 0.26.

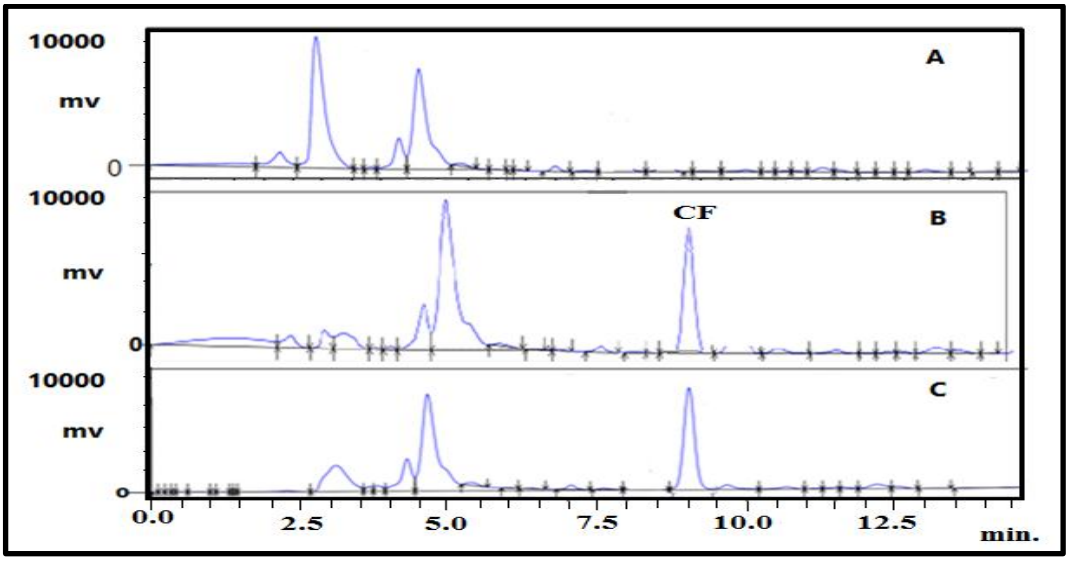

Figure 3: Chromatograms recorded at chromatographic conditions were RP-C18 as stationary phase, mixture of methanol:water (95:5 v/v) as mobile phase, flow rate of 0.8 mL/min, at room temperature and fluorescence detection at λem:280 nm , and λex: 340 nm. A: Blank of tomato B: CF (Chlorpyrifos) standard solution (70.0 µg/mL), C: Spiked tomato at concentration 10.0 (µg/g).

Under chromatographic conditions a concentration of 0.01 (µg/g) was not achieved –MRL of CF in tomato - although extract was condensed before analysis.

Accuracy

The accuracy of the method was evaluated by recovery studies of CF at method.

three different chlorpyrifos concentration (0.05, 1.0, and 5.0 µg/g).

Percent recovery is listed in table 2, which ranges from 77.5 to 109.4%. Results revealed good accuracy for the proposed

Table 1: Linearity data of CF.

Parameter	Result
Matrix matched calibration curve*	Y = (724169 ± 2.3)X + (459043 ± 10.8)**
Correlation coefficient R^{2*}	0.9964 ± 0.26
Range (µg/g)	0.05-10.0
LOD (µg/g)	0.01
LOQ (µg/g)	0.05

*: Results represent the mean ± % SD for three determinations, **: Y: Area of peak, X: CF concentration (µg/g)

Table 2: Results of recovery studies.

Spiked CF Conc. (µg/g)	Conc. calc. (µg/g)*	% Recovery
0.05	0.04	77.5
1.0	0.87	87.3
5.0	5.60	109.4
*: Concentration of CF calculated by matrix matched calibration curve.		

Precision

Intra-day precision was assessed by analyzing three replicates of solutions during one day. Inter-day precision was assessed by analyzing three different concentrations on three days within a week. The results are given in table 3.

The percent of relative standard deviation RSD were less than 20% for intra- and inter-day precision, which were within the acceptable criteria (European commission, 2002).

Table 3: Results of intra-day and inter-day precision (n = 3).

Spiked CF Conc. (µg/g)	% RSD*	
	Intra-day	Inter-day
0.05	18.4	19.2
1.0	16.2	15.8
5.0	8.9	11.4
*: RSD is relative standard deviation.		

Analysis of tomato samples

The results of collected tomato samples are given in table 4. Sampling and subsampling procedure followed the recommended guidelines to have a representative sample for analysis (FAO, 1988).

Table 4: Results of tomato sample analysis on residual CF.

Origin of sample	Average area (n = 2)	Conc. (µg/g)
Rafah	ND	--------
Khanyounis	ND	---------
Deir El-Balah	ND	---------
Shatee	4450084	5.51
Beit Lahia	1364816	1.25
Beit Hanoun	3669294	4.43
ND: Not detected at retention time of CF under chromatographic conditions.		

CF was detected in three (out of six) samples at concentrations ranged from 1.25 to 5.51 (µg/g), which was above the MRL. Detection of CF residues needs further a confirmatory test using more sophisticated instruments like HPLC-MS-MS (European commission, 2021). Mass spectrophotometer is the advanced detector for ultra-traces analysis in different matrices since the targeted analyte can be detected even in the presence of coeluted substances (Song et al., 2020). This result showed that pesticides have been used indiscriminately (FAO, 2016), which could lead to health problems not only to the farmers but also to the general consumers (Blair, 2014). Health hazards posed by pesticides ranged from short term impacts like headache, and nausea to long term impacts like cancer, endocrine disruption, and reproductive harm (Chen & Qian, 2011). The presence of pesticide residue in food, especially when fruit are consumed fresh, consisting of more than 30% of consumer diet is highly associated with residues in human body (Kazar Soydan *et al.*, 2021). To reduce pesticide residue in food good agricultural practices (GAP) is a valuable issue to be followed. GAP produce safe and healthy food while considering economic, social, and environmental sustainability (FAO, 2003). To reduce pesticide contamination of fresh agricultural products, simple steps e.g. boiling, washing, and peeling should be followed by consumers (Ramadan *et al.*, 2016).

Detection of pesticide residue in vegetables marketed and cultivated in the Gaza Strip was reported in the literature (Safi *et al.*, 2002, Safi *et al.*, 2001, Batta, 2003, Al-Sa'ed *et al.*, 2011, El-Nahal, 2004, El-Nahal and Safi, 2012).

Since the aim of study was to evaluate the analytical process of ultra-trace residue in our local labs depending on available instrument and chemicals, analysis of real samples was performed after completeness of the verification process (European Commission, 2021). Results reflected the contamination only of the tested samples. To have a better overview about food contamination with pesticide in the Gaza Strip further studies using more advanced instrument, higher number of samples, and various samples collected from different farms on different times within the year are required. The routine analysis of agricultural products is still facing many obstacles in the Gaza Strip-Palestine, which is a problem that needs a strategic solution.

Conclusion

The present work described the efficient application of QuChERs extraction procedure. HPLC coupled with fluorescence detection was used to analyze residual chlorpyrifos (CF) in tomato. CF was detected in 50% of collected samples. The results of this study – even recorded for one time and lack the confirmatory testing - should be considered by regulatory authorities to follow up the agricultural practices among farmers and perform routine quality control testing on possible food contamination. Many obstacles are facing residual pesticide analysis in the

Gaza Strip due to uncertainty of solvent quality, lack of chromatographic instruments coupled with MS detector, and the high cost of materials. Establishment of well-equipped labs and training of lab technicians on ultra-traces analysis is advised for research centers and regulatory authorities in the Gaza Strip. Further studies on pesticide residue in vegetables are highly recommended.

Acknowledgment

The authors would like to thank Qatar Red Crescent for equipping the Drug and Toxicology Analysis Centre with modern instruments.

References

Akhtar, N., Srivastava, M. K., & Raizada, R. B. (2009). Assessment of chlorpyrifos toxicity on certain organs in rat, Rattus norvegicus. *Journal of Enviromental Biology*, *30*(6), 1047-1053. http://www.jeb.co.in/journal_issues/200911_nov09/paper_20.pdf

Al-Kurdi, S., Alloh, M. O., Al-Agha, M. R., & El-Nahhal, Y. (2018). Development of analytical method for the detection of Nemacur residues in cucumber fruits. *American Journal of Analytical Chemistry*, *9*(1), 64-76. https://www.scirp.org/journal/paperinformation.aspx?paperid=81968

Al-Sa` ed, R., Ramlawi, A. D., & Salah, A. (2011). A national survey on the use of agricultural pesticides in Palestine. *International journal of environmental studies*, *68*(4), 519-529. https://www.tandfonline.com/doi/abs/10.1080/00207233.2011.608502

ARIJ. (2020) Palestine's contaminated vegetables: Present on our tables, absent regulations. The International Development Research Centre (IDRC), West Bank-Palestine. https://arij.net/investigations/toxic-pesticides-en/

Batta, Y. (2002) Subject Review: Application and usage of pesticides in Palestine: Current and future outlook. An-Najah University Journal for Research-A (Natural Sciences), 17(1), 89-98. https://journals.najah.edu/article/645/

Blair, A. (2014). Pesticide and human health. *Occupational and Environmental Medicine, 72,* 81-82.

Capriotti, A. L., Cavaliere, C., Laganà, A., Piovesana, S., & Samperi, R. (2013). Recent trends in matrix solid-phase dispersion. *Trends in Analytical Chemistry*, *43*, 53-66.

https://www.sciencedirect.com/science/article/pii/S0165993612003111

Casida, J. E., Bryant, R. J. (2017). The ABCs of pesticide toxicology: amounts, biology, and chemistry. *Toxicology Research (Camb). 22, 6(6),* 755-763. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6062263/

Chen, C., and Qian, Y. (2011). Evaluation of pesticide residues in fruits and vegetables from Xiamen, China. *Food control, 22*, 1114- 1120.

Codex Alimentarius Commission. (2020) International food standards. Codex pesticide, commodities detail, VO 0448-tomato. https://www.fao.org/fao-who-codexalimentarius/codex-

texts/dbs/pestres/commodities-detail/en/?c_id=320

Codex Alimentarius Commission. (2007). *International Food Standards*. Pesticide residues in food and feed. Codex pesticides residues in food online database. https://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/pesticide-detail/en/?p_id=17.

Commission of the European Communities.(2002) Commission directive 2002/27/EC amending Directive 98/53/EC laying down the sampling methods and the methods of analysis for the official control of the levels for certain contaminants in foodstuffs. Official Journal of European Communities. L75/44-45. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:075:0044:0045:EN:PDF

Dorais, M., Ehret, D. L., & Papadopoulos, A. P. (2008). Tomato (Solanum lycopersicum) health components: from the seed to the consumer. *Phytochemistry Reviews*, *7*(2), 231-250.

https://link.springer.com/article/10.1007/s11101-007-9085-x

El-Nahhal, Y. (2004). Contamination and safety status of plant food in Arab countries. Journal of Applied Sciences, 4(3), 411-417. https://iugspace.iugaza.edu.ps/handle/20.500.12358/26207

El-Nahhal, Y. & Safi, J. (2012) Removal of organic pollutants from water by modified bentonite. Pesticides-Advances in Chemical and Botanical Pesticides, Chapter 5, 93-102.

https://books.google.com/books?hl=en&lr=lang_en&id=GEueDwAAQBAJ&oi=fnd&pg=PA75&dq=+Removal+of+Organic+Pollutants+from+Water+by+Modified+Bentonite.+Pesticides

El-Najar, H. M. M. (2001). The monitoring of pesticide residues in fruits and vegetables in the Gaza governorates (pesticide residue analysis in cucumbers, tomatoes, strawberries and grapes). AGRIS, iv, 82p. https://agris.fao.org/agris-search/search.do?recordID=QC20042004992.

European Commission. (2021). Guidance document on pesticide analytical methods for risk assessment and post-approval control and monitoring purposes. SANTE/2020/12830-rev. 1. https://ec.europa.eu/food/system/files/2021-02/pesticides_mrl_guidelines_2020-12830.pdf

FAO. (2016). A scheme and training manual on good agricultural practices GAP for fruits and vegetables. Food and agriculture organization of the United Nations. https://www.fao.org/3/i6677e/i6677e.pdf

FAO. (1988). Manuals of food quality control 9. Introduction to food sampling. Food and agriculture organization of the United Nations.

FAO. (2003). Report of the FAO Expert Consultation on a Good Agricultural Practice Approach, Rome. FAO Agricultural Department Report Food and agriculture organization of the United Nations.

Gupta, R. (2011) Reproductive and developmental toxicology. Pages 503-521.

Hazer, O., Akkbik, M., Demir, D., & Turhan, Y. (2017). Determination of carbendazim and chlorpyrifos in selected fruits and vegetables samples using QuEChERS-HPLC-FD. *Eurasian Journal of Analytical Chemistry*, *12*(2), 17-30. https://avesis.bozok.edu.tr/yayin/d1845470-6b77-4219-869d-27c0b2ba081c/determination-of-carbendazim-and-chlorpyrifos-in-selected-fruits-and-vegetables-samples-using-quechers-hplc-fd

Hercegová, A., Dömötörová, M., & Matisova, E. (2007). Sample preparation methods in the analysis of pesticide residues in baby food with subsequent chromatographic determination. *Journal of Chromatography A*, *1153*(1-2), 54-73.

Kazar Soydan, D., Turgut, N., Yalçın, M., Turgut, C., & Karakuş, P. B. K. (2021). Evaluation of pesticide residues in fruits and vegetables from the Aegean region of Turkey and assessment of risk to consumers. *Environmental Science and Pollution Research*, *28*(22), 27511-27519.

Kim, L., Lee, D., Cho, H. K., & Choi, S. D. (2019). Review of the QuEChERS method for the analysis of organic pollutants: Persistent organic pollutants, polycyclic aromatic hydrocarbons, and pharmaceuticals. *Trends in Environmental Analytical Chemistry*, *22*, Article e00063. https://www.sciencedirect.com/science/article/pii/S0021967307000350

Lawal, A., Wong, R. C. S., Tan, G. H., Abdulra'uf, L. B., & Alsharif, A. M. A. (2018). Recent modifications and validation of QuEChERS-dSPE coupled to LC–MS and GC–MS instruments for determination of pesticide/agrochemical residues in fruits and vegetables. *Journal of Chromatographic Science*, *56*(7), 656-669. https://academic.oup.com/chromsci/article-abstract/56/7/656/4980918

Lewis, K., & Tzilivakis, J. (2017). Development of a data set of pesticide dissipation rates in/on various plant matrices for the pesticide properties database (PPDB). *Data*, *2*(3), 28. https://www.mdpi.com/220358

Medina-Pastor, P., & Triacchini, G. (2020). The 2018 European Union report on pesticide residues in food. *EFSA Journal*, *18*(4), Article e06057.

https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2020.6057

Mohammed, S., Lamoree, M., Ansa-Asare, O. D., & de Boer, J. (2019). Review of the analysis of insecticide residues and their levels in different matrices in Ghana. *Ecotoxicology and Environmental Safety*, *171*, 361-372. https://www.sciencedirect.com/science/article/pii/S014765131831337X

Narenderan, S. T., Meyyanathan, S. N., & Babu, B. J. F. R. I. (2020). Review of pesticide residue analysis in fruits and vegetables. Pre-treatment, extraction and detection techniques. *Food Research International*, *133*, Article 109141.

https://www.sciencedirect.com/science/article/pii/S0963996920301666

PCBS (Palestinian Central Bureau of Statistics) (2010). Dissemination and analysis of Agricultural Census. https://www.pcbs.gov.ps/Downloads/book1839.pdf

Perestrelo, R., Silva, P., Porto-Figueira, P., Pereira, J. A., Silva, C., Medina, S., & Câmara, J. S. (2019). QuEChERS-Fundamentals, relevant improvements, applications and future trends. *Analytica chimica acta*, *1070*, 1-28. https://www.sciencedirect.com/science/article/pii/S0003267019302259

PPDB (Pesticide Properties Data Base) (2020). https://comptox.epa.gov/dashboard/chemical-lists/PPDB

Ramadan, G., Shawir, M., El-Bakary, A., & Abdelgaleil, S. (2016). Dissipation of four insecticides in tomato fruit using high performance liquid chromatography and QuEChERS methodology. *Chilean journal of agricultural research*, *76*(1), 129-133. https://www.scielo.cl/scielo.php?pid=S071858392016000100018&script=sci_arttext&tlng=pt

Rejczak, T., & Tuzimski, T. (2015). A review of recent developments and trends in the QuEChERS sample preparation approach. *Open Chemistry*, *13*(1), 980-1010. https://doi.org/10.1515/chem-2015-0109

Safi, J. M., Abou-foul, N. S., El-Nahhal, Y., & El-sebae, A. H. (2001). Monitoring of pesticide residues on green pepper, potatoes, vicia faba, green bean and green peas in Gaza Governorates, Palestine. *Journal of Pest Control and Environmental Sciences, 9*(1), 55-72.

Safi, J. M., Abou-Foul, N. S., El-Nahhal, Y. Z., & El-Sebae, A. H. (2002). Monitoring of pesticide residues on cucumber, tomatoes and strawberries in Gaza Governorates, Palestine. *Food/Nahrung*, *46*(1), 34-39.

Samsidar, A., Siddiquee, S., & Shaarani, S. M. (2018). A review of extraction, analytical and advanced methods for determination of pesticides in environment and foodstuffs. *Trends in Food Science & Technology*, *71*, 188-201. https://www.sciencedirect.com/science/article/abs/pii/S0924224417303783

Song, N. E., Jung, Y. S., Choi, J. Y., Koo, M., Choi, H. K., Seo, D. H., Lim, T-G & Nam, T. G. (2020). Development and application of a multi-residue method to determine pesticides in agricultural water using quechers extraction and LC-MS/MS analysis. *Separations*, *7*(4), Article 52. https://www.mdpi.com/846200

Stachniuk, A., Szmagara, A., Czeczko, R., & Fornal, E. (2017). LC-MS/MS determination of pesticide residues in fruits and vegetables. *Journal of Environmental Science and Health, Part B*, *52*(7), 446-457. https://www.tandfonline.com/doi/abs/10.1080/03601234.2017.1301755

Tankiewicz, M. (2019). Determination of selected priority pesticides in high water fruits and vegetables by modified QuEChERS and GC-ECD with GC-MS/MS confirmation. *Molecules*, *24*(3), Article 417. https://www.mdpi.com/400822

Wahab, S., Muzammil, K., Nasir, N., Khan, M. S., Ahmad, M. F., Khalid, M, Ahmed, W., Dawria, A., Reddy, L. V. & Busayli, A. M. (2022). Advancement and new trends in analysis of pesticide residues in food: A comprehensive review. *Plants*, *11*(9), 1106. https://www.mdpi.com/2223-7747/11/9/1106

Yahya, A. (2021). Pesticide residues in imported sweet peppers, tomatoes and

grapes in the north West Bank. *Dissertation thesis.* Al-Najah National University. https://repository.najah.edu/handle/20.500.11888/16469

Yuan, Y., Chen, C., Zheng, C., Wang, X., Yang, G., Wang, Q., & Zhang, Z. (2014). Residue of chlorpyrifos and cypermethrin in vegetables and probabilistic exposure assessment for consumers in Zhejiang Province, China. *Food Control*, *36*(1), 63-68. https://www.sciencedirect.com/science/article/pii/S0956713513004003