



# Improved analysis of folpet and captan in foods using liquid chromatography-triple quadrupole linear ion trap mass spectrometry: applying mass filtering, collision, and trapping conditions

Abd-Allah A. El-Sheikh<sup>1,2</sup> · Osama H. Elhamalawy<sup>2</sup> · Sherif M. Taha<sup>1</sup> · Fawzy I. Eissa<sup>2</sup>

Received: 17 January 2023 / Revised: 17 March 2023 / Accepted: 21 March 2023 / Published online: 11 April 2023  
© Springer-Verlag GmbH Germany, part of Springer Nature 2023

## Abstract

Accurate and highly sensitive analysis of folpet and captan was accomplished using liquid chromatography-triple quadrupole linear ion trap mass spectrometry (LC-QqQIT) with selective ion mode; mass filtering, collision, and trapping condition. Dimensional mass spectrometry (MS<sup>3</sup>) parameters were optimized for the residue detection of folpet and captan in six food commodities (apples, tomatoes, sweet pepper, wheat flour, sesame seeds, and fennel seeds). The sample preparation method was based on the known QuEChERS protocol, except a mixture of acetonitrile/acetone was used for the sample extraction from the sesame seeds. The robustness and reliability of the developed MS<sup>3</sup> method were demonstrated by performing a full validation, according to SANTE/11312/2021, at 0.01–0.25 mg/kg. Recovery ranged from 83 to 118% with a relative standard deviation below 19% in all the tested commodities, and limits of quantifications (LOQs) were 0.01 mg/kg in apples and tomatoes; 0.03 mg/kg in sweet pepper; and 0.05 mg/kg in wheat flour, sesame seeds, and fennel seeds. Monitoring results showed that about 90% of apples contained captan residue, and in sweet pepper, concentrations of captan and folpet as high as 1.57 and 0.97 mg/kg were found, respectively. The novel developed MS<sup>3</sup> method enabled more reliable identification of these commonly problematic fungicides at lower LOQs than previously reported methods.

**Keywords** Folpet · Captan · LC-QqQIT · QuEChERS · Linear ion trap · MS<sup>3</sup>

## Introduction

The average worldwide pesticide usage increases annually with the increased demand for food production [1]. Folpet is a non-systemic fungicide that is commonly used for the treatment of several agricultural products [2] like grapes, stone fruit, and vegetables [3]. Captan is a contact fungicide that controls and protects a variety of crops from a broad range of diseases [4]. These fungicides have nearly similar structures and belong to the thiophthalimide family [5], with an aromatic ring for folpet. They were used as fungicides for several decades [6] and their usage may be extended

for several future decades. However, their maximum residue limits (MRLs) in different agricultural products are not as low as needed and are mainly related to the reported limits of their quantitation using the available testing methods [7]. This is attributed to the inability to determine lower concentrations for these fungicides using routine multi-residue pesticide analysis. Furthermore, there are various difficulties in their residue determination in food using specific single-residue methods, as they are degraded under different conditions: light, basic pH, hydrolysis, and heat [8]. Therefore, folpet and captan analysis using the common standard sample preparation protocol, the most known QuEChERS method, was accomplished using cold water, acidified MeCN, and avoiding any pH increase (no clean-up with primary-secondary amine (PSA)) [9, 10].

In the known tandem mass spectrometry (QqQ), the three consecutive quadrupoles are working in two modes: mass filters (both the first and third one) and ion collision (the second quadrupole). These systems enable more accurate detection of a compound through multiple reaction transition (MRM) experiments. Folpet and captan are degraded

✉ Sherif M. Taha  
sherif2taha@gmail.com

<sup>1</sup> Agricultural Research Center, Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Foods, Dokki, P.O. Box 12311, Giza, Egypt

<sup>2</sup> Environment and Bio-Agriculture Department, Faculty of Agriculture, Al-Azhar University, P.O. Box 11884, Cairo, Egypt

inside the different parts of the heated gas chromatography-tandem mass spectrometry (GC-QqQ) system into phthalimide (PI) and tetrahydrophthalimide (THPI), respectively [11, 12]. Therefore, they may be indirectly determined by the analysis of these degradation products. Therefore, the EU definitions of folpet and captan were extended to include their degradation products [13, 14]. However, the percent of folpet and captan degradation into PI and THPI is not constant [8], especially in the routine sample analysis using the GC-QqQ technique [15], where these compounds were largely adsorbed and degraded in the heated linear, column, and on the surface of the detector. Furthermore, degradation of folpet and captan may occur during the sample processing (grinding) or sample preparation, especially when a basic dispersive solid phase extraction salt is used. In addition, PI and THPI can be found as a degradation product from other pesticides such as ditalimphos, phosmet, and captafol [16].

On the other hand, the lower sensitivity of folpet and captan was obtained when using LC-QqQ [17], especially when they were detected in complex food matrices. Although previous studies optimized specific LC-QqQ mass ionization parameters improving the sensitivity of folpet and captan [18], the obtained results are not satisfactory in terms of peak shape, sensitivity, and selectivity [19]. On the other hand, it was reported that some specific techniques enable better residue detection of folpet and captan like supercritical fluid chromatography-tandem mass spectrometry (SFC-QqQ) [17], atmospheric pressure chemical ionization [20], negative chemical ionization GC-QqQ techniques [21], GC-electron capture detection (GC-ECD) [22], and an on-column injection GC-QqQ method [16]. However, these specific techniques are not widely present and some of them have limitations in routine analyses. Therefore, the development of a method that enables the direct instrumental determination of folpet and captan at lower concentrations in various food commodities using commonly present instruments in food safety laboratories is in high demand.

A hybrid quadrupole system (LC-QqQIT) employs the usage of the first quadrupole as a mass filter, the second as a collision cell, and the third as a mass filter, ion collision, and linear ion trap (LIT). LC-QqQIT is quite different from the commonly known hyphenated system LC-QqQ system. Recently, LC tandem mass spectrometer techniques can be used as LC-QqQ and also as LC-QqQIT, enabling MRM and MS<sup>3</sup> modes of analyses, respectively. LIT has a higher ion trapping volume and therefore has a higher ion focusing efficiency than the three-dimensional traps [23]. Additional advancements in recent available LC-QqQIT technologies, in light of scan rate, mass resolution, and other mass parameters, enable the efficient application of MS<sup>3</sup> mode in routine residue analysis of various contaminants. Using ion trap technology was previously reported for the residue determination of moderate to high mass compounds like

tachykinin-related peptides in the spinal cord of rats [24] and monomethyl-arginine in yeast [25].

In the current study, the LC-QqQIT technique is used to enable the accurate and sensitive residue analysis of both folpet and captan in different food commodities. Several MS<sup>3</sup> transitions were optimized for the identification and quantification of residue analysis of the studied fungicides. Furthermore, the developed MS<sup>3</sup> and the classical MRM methods of folpet and captan were compared for their residue analysis in the studied food commodities. Finally, the developed MS<sup>3</sup> method was validated in different food commodities, including apples, sweet pepper, tomatoes, wheat flour, and sesame seeds according to the EU validation guidelines, SANTE/11312/2021 [26].

## Materials and methods

### Chemicals and reagent

Methanol was supplied by Fisher Scientific. Ammonium hydroxide reagent and formic acid were purchased from Merck. Ultrapure de-ionized water (DIW) was prepared using a Milli-Q water purification system (Merck). Ready-prepared QuEChERS extraction salt mixture (EN 15,662) containing 4 g MgSO<sub>4</sub>, 1 g NaCl, 1 g Na-citrate, 0.5 g Na<sub>2</sub>-citrate sesquihydrate was purchased from Agilent Technologies. Active reference standards of folpet and captan were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Individual stock standard solutions were prepared in pure acetonitrile and kept at -20 °C, then working standard solution mixtures were prepared in acetonitrile containing 0.1% formic acid, kept at 4 °C.

### Sample preparation

Various food commodities, including apples, sweet pepper, and tomatoes (fresh produce); fennel seeds (herbal plants); wheat flour (high-protein and dry samples); and sesame seeds (high oil and protein contents), were collected from local Egyptian markets. A blank test sample representing each of these commodities was used in performing the method validation.

Fresh produce samples were firstly cut into small pieces, then frozen at -80 °C. This portion was ground in a Knife Mill Grindomix (GM 300), using dry ice. The dry samples were also directly ground using dry ice. Ten grams of fresh samples and 2 g of dry samples were weighed in a 50-ml polypropylene conical centrifuge tube. After that, 10 ml of DIW was added only to fennel seeds and wheat flour samples which were subsequently shaken for 1 min at 500 rounds per minute (rpm) in an automatic axial agitator (Geno/Grinder). Next, 10 ml of acidified acetonitrile (1% formic

acid) was used for the extraction of folpet and captan from all the studied samples, except sesame seeds, which were extracted directly using 10 ml of acidified (1% formic acid) acetonitrile/acetone mixture (9:1, v:v). Tubes corresponding to the dry samples were placed in a freezer, for 1 h, before executing the next solvent phase-out step which involves the usage of magnesium sulfate that markedly increases the sample temperature, especially diluted dry samples [27], and subsequently affect the stability of folpet and captan. The phase-out step was accomplished using ready-prepared QuEChERS extraction salt and then shaken for 1 min at 500 rpm by Geno/Grinder. Then, the tubes were centrifuged for 5 min at 4500 *ref*. Finally, about 1.0 ml of the upper layer was filtered through a PTFE syringe filter (diameter and pore size of 25 mm and 0.45  $\mu\text{m}$ , respectively) and transferred into a brown glass vial to be ready for the direct analysis using LC-QqQ and LC-QqQIT systems, applying MRM and  $\text{MS}^3$  transitions, respectively.

### Instrumental analysis

A Shimadzu HPLC system (Exion LC) with C18 column (pro-shell 120, 3 mm  $\times$  50 mm  $\times$  2.7  $\mu\text{m}$ ) was used for the separation of the target analytes. The mobile phase components consisted of (A) solvent: a mixture of DIW:MeOH (9:1, v:v) of pH 4, using ammonium formate; and (B) solvent: MeOH. The mobile phase was pumped at a flow rate of 0.4 ml/min with an optimized solvent gradient program as follows: 0–1 min; 30% B, 1.1–4 min; a linear gradient to 95% B; remained fixed at this condition to 7 min, 7.1–10 min; returned to 30% B. The eluted times of captan and folpet were 4.23 min and 4.54 min, respectively. These chromatographic conditions result in well-separated peaks of folpet and captan in complex matrices that were prepared without applying a clean-up step.

AB SCIEX 6500 plus equipped with an IonDrive Turbo V source was used in this study. This technique can be used as LC-QqQ and LC-QqQIT. It was operated in the soft positive electrospray ionization (ESI) mode. An automated mass infusion was applied to optimize mass parameters of the most sensitive MRM transitions for folpet and captan. These main optimized mass parameters include declustering potential (DP), entrance potential (EP), collision energy (CE), and collision-exit potential (CEX). Furthermore, additional mass parameters including ion-spray voltage, ion source temperature, nebulizing gas (gas 1), and drying gas (gas 2) were manually optimized. The most sensitive MRMs were considered for the subsequent development of  $\text{MS}^3$  transitions using LC-QqQIT. Where each of the selected MRMs was first further tested using MRM-scan mode by slightly fragmenting the second molecule (first fragmented in the second quadrupole) into the third quadrupole and obtaining a full mass spectrum. Then after, the most sensitive second

fragment was selected, and construct  $\text{MS}^3$  that being further improved using additional mass conditions.

Ions focusing/trapping at the first mass filtering quadrupole (Q0) was also applied to further enhance the selectivity and sensitivity of detected ions before it enters the main set of quadrupoles in the QqQIT system. The collision-associated dissociation (CAD) was also used at high mode to increase ion focusing and trapping in the last quadrupole, which works as a quadrupole ion trap (QIT) at  $\text{MS}^3$  testing mode.

### Method validation

Full validation was carried out on apples in terms of accuracy, precision, limits of quantitation (LOQs), linearity, and matrix effect. The accuracy and precisions were determined by calculating the recovery (Rec) and relative standard deviation (RSD) of six fortified spikes at three concentrations of 0.01, 0.02, and 0.05 mg/l (equivalent to the same concentrations in apples, mg/kg) on the same day, intra-day precision. The inter-day precision was calculated using five fortified spikes at 0.02 mg/kg, over five consecutive days. The linearity was evaluated using a set of six matched calibration levels (0.005, 0.01, 0.02, 0.05, 0.10, and 0.5 mg/l) that were prepared in a blank apple extract.

Additional fortification spikes ( $n=6$ ) in tomatoes and sweet peppers were performed at concentrations of (0.01 and 0.05 mg/l) and (0.03 and 0.05 mg/l), respectively, equivalent to the same concentrations in these samples, mg/kg. Furthermore, fortification spikes ( $n=6$ ) were carried out in fennel, wheat flour, and sesame seeds at concentrations of 0.01 and 0.05 mg/l (equivalent to concentrations of 0.05 and 0.25 mg/kg in these dry samples).

### Matrix effects calculation

The matrix effects (ME) of folpet and captan were calculated using the following equation:

$$\text{ME \%} = \left( \frac{\text{Slope of pesticide calibration in A}}{\text{Slope of pesticide calibration in solvent}} - 1 \right) * 100 \quad (1)$$

A, blank sample extract (apples, sweet pepper, tomatoes, wheat flour, fennel, and sesame).

## Results and discussion

### Sample preparation

Sample processing was carried out as previously reported using dry ice [17]. The pore size of the used syringe filter was 0.45  $\mu\text{m}$ , and a lower mesh volume was avoided [28]

to minimize any adsorption of folpet and captan inside the used narrow filter. No clean-up was performed neither using solid phase extraction columns nor dispersive solid phase salts to minimize any loss in folpet and captan during sample preparation.

### Improved analysis of folpet and captan using LC-QqQIT

Ionization of folpet and captan were performed using the soft positive ESI mode at the following parameters; ion spray voltage of 5500 v, the temperature of 250 °C, and values gas 1 and 2 are 50 µl/min. The optimized mass transitions (nine MRM and six MS<sup>3</sup>) based on the properly developed mass ionization, filtering, and trapping parameters, for the identification and quantifications of folpet and captan in the studied samples, are presented in Table 1. The effect of the main optimized mass parameters on the MS<sup>3</sup> sensitivities is shown in Fig. 1. It is known that better ion focusing and the subsequent ion transmission towards the set of QqQIT are accomplished when using a radiofrequency-multipole (RF) [29]. Therefore, when permitting the usage of the ion trapping/focusing on the first ion guide (q0), the sensitivity of the selected MS<sup>3</sup> was enhanced at least tenfold, as shown in Fig. 1A and B. Furthermore, using the buffer gas (CAD) at a high level permits a higher ion focusing (to both the precursor and fragmented ions) and subsequently improves the sensitivities (Fig. 1C). Finally, increasing the trapping time of the

selected fragment ion (inside the third quadrupole, QIT) permits higher sensitivities and higher signal-to-noise ratio of the selected MS<sup>3</sup>, as shown in Fig. 1D. These results reflect the importance of the filling time that does not only permit a further improvement in the ion focusing but also largely minimize the effect of the co-extracting interfering masses.

The chromatograms of folpet and captan using MS<sup>3</sup> and those using MRM in apples at a conc. of 0.01 mg/kg are shown in Fig. 2. As shown, the folpet and captan analysis using MS<sup>3</sup> results in much higher sensitivity by an average of 10 e<sup>6</sup> times than those related to the best optimized MRM. On the other hand, it was elucidated that residue analysis of folpet and captan in wheat flour and sweet pepper cannot be accomplished using MRM transitions, especially at a lower conc. of 0.02 mg/kg, as shown in Figs. 3 and 4, respectively. The same results were obtained for the residue analysis of folpet and captan at a low conc. of 0.01 mg/kg in sesame and fennel seeds, as shown in Figs. S1 and S2, respectively.

These results clearly emphasize that the optimized MS<sup>3</sup> transitions of folpet and captan are more sensitive and selective (main identification requirements for different MS techniques, SANTE/11312/2021) than MRM transitions even at lower concentration levels in different agricultural products, including complex matrices like wheat flour, sweet pepper, and sesame seeds.

### Method validation

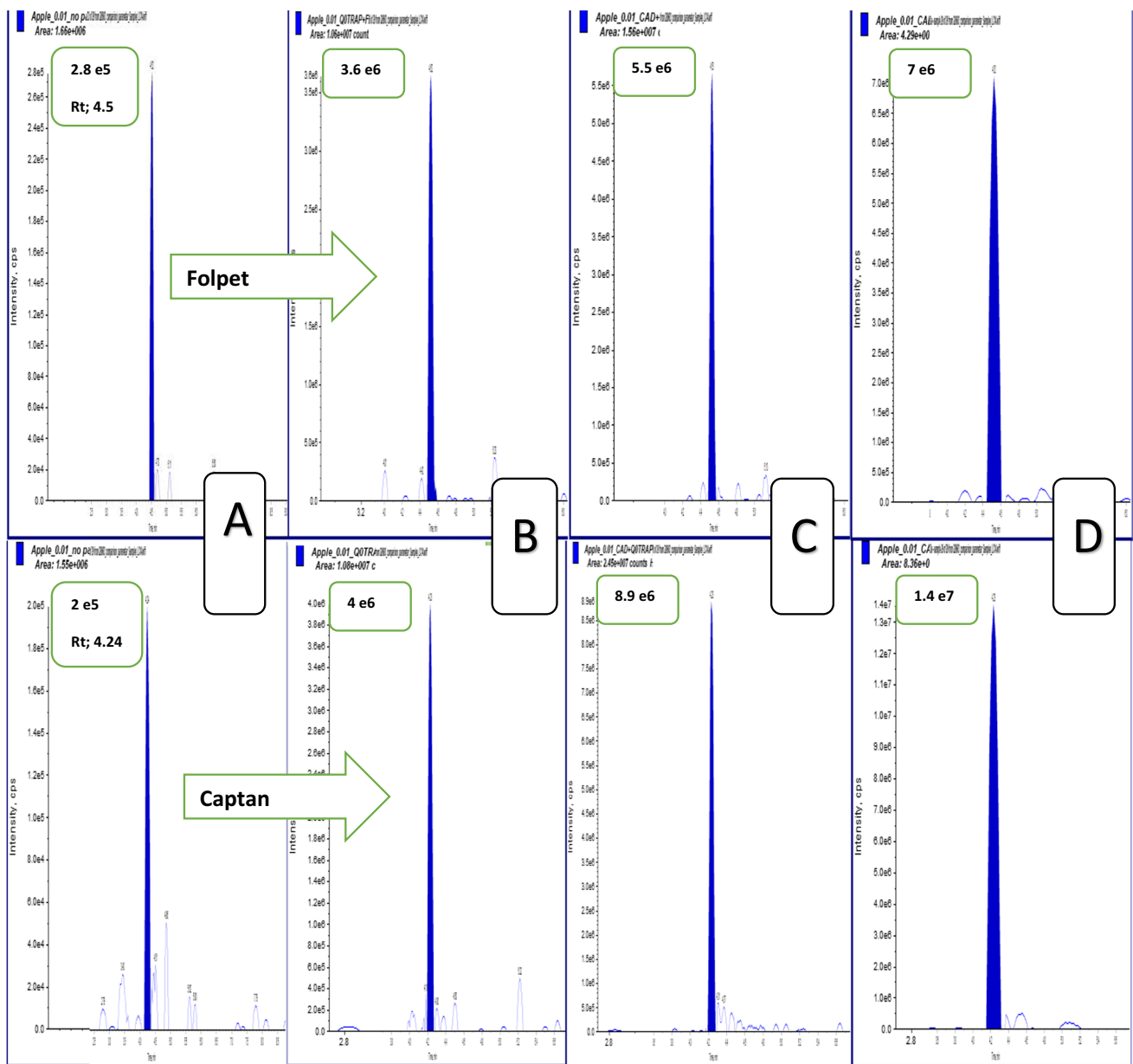
The analysis of folpet and captan using the final optimized MS<sup>3</sup> method was fully validated in apples, according to the European guidelines, SANTE/11312/2021. Table 2 presents the validation parameters for the selected three MS<sup>3</sup> transitions of folpet and captan in apples at 0.01, 0.02, and 0.05 mg/kg. The highly accurate and precise results (Rec between 83 and 111%, with RSD ≤ 17%) indicate the efficient quantitative detection of folpet and captan in apples using the developed MS<sup>3</sup> method. The lowest validation level of 0.01 mg/kg that meets the main identification criteria (accuracy and precision) was considered as LOQ, SANTE/11312/2021. This concentration was much lower than the published EU-MRLs of folpet and captan in apples.

Calculated Rec and RSD for a set of six fortified spikes of folpet and captan in various food commodities at different concentrations including tomatoes (0.05 mg/kg), sweet pepper (0.03 and 0.05 mg/kg), and wheat flour, sesame seeds, and fennel seeds (0.50 and 0.25 mg/kg) are presented in Table 3. Acceptable recoveries with low RSD values were obtained for these measurements also emphasizing the rigidity of applying this method for

**Table 1** Main mass transition (MRM and MS<sup>3</sup>) parameters for the residue analysis of captan and folpet LC-QqQ and LC-QqQit

Compound	No	MRM	DP	CE	CXP
Captan	1	318.9*-301.9	61	9	16
	2	318.9*-265.9	61	17	16
	3	318.9*-263.9	61	17	30
	4	318.9*-238	61	23	12
	5	300-263.5	61	23	12
Folpet	1	312.9*-259.8	21	15	16
	2	312.9*-130	21	33	14
	3	312.9*-295.9	21	9	16
	4	314.9*-262	21	9	16
Compound	No	MRM <sup>3</sup>	DP	CE	Fill time, ms
Captan	1	300-263.5-236	61	10	150
	2	302-265.5-238	61	10	150
	3	319*302-238	61	9	150
Folpet	1	313*-259.8-130	21	9	150
	2	313*-130-102	21	10	150
	3	315*-262-234	21	15	150

\*Ammonium adduct



**Fig. 1** The analysis of folpet and captan (0.01 mg/kg) in apple using LC-QqQIT at different conditions; A) without trapping at Q0, applying collision associate dissociation (CAD) at low, and QIT with a dynamic fill time, B) ion trapping in Q0, low CAD and a fill time of

20 ms in QIT, C) ion trapping in Q0 trap, high CAD and fill time of 20 ms in QIT, D) the final optimized LC-QqQIT method; ion trapping in Q0 trap, high CAD and fill time of 150 ms in QIT

the residue analysis of folpet and captan in different food matrices.

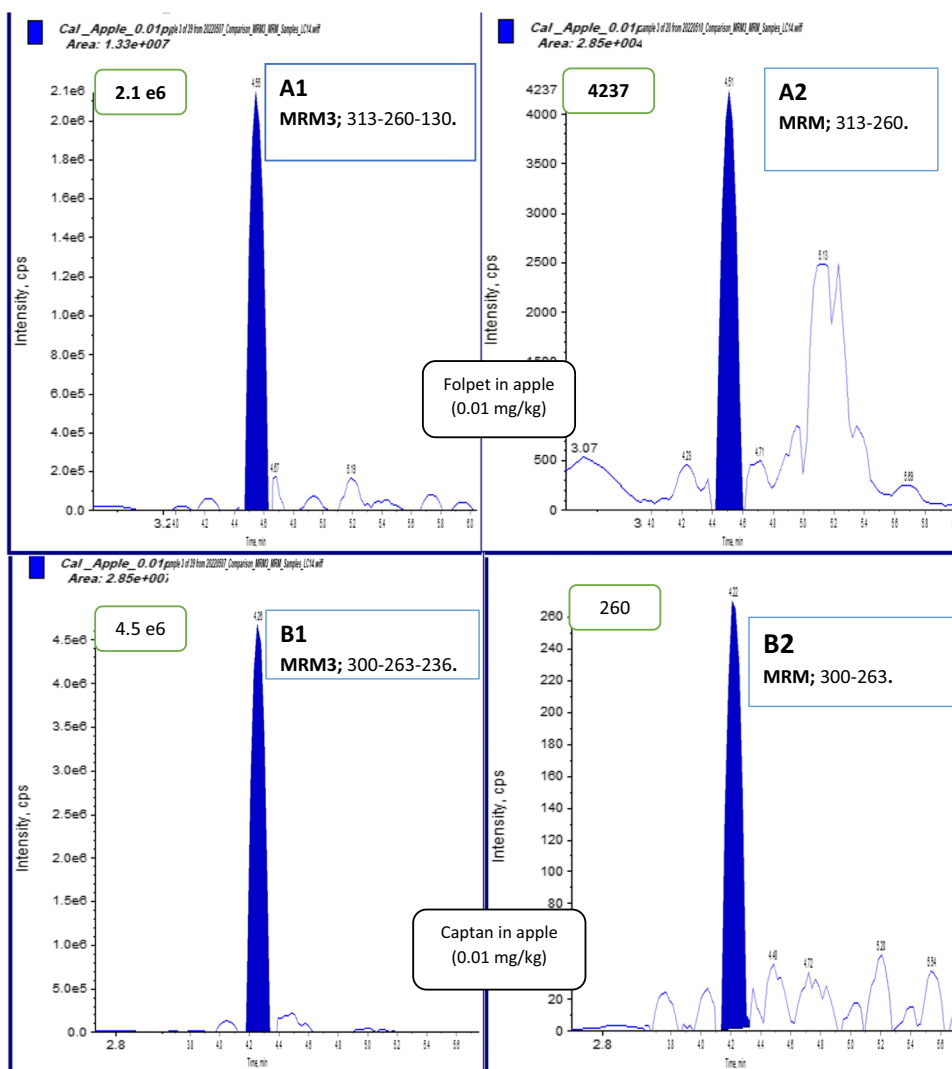
### Linearity and matrix effects

The linearity of captan and folpet analyses using the developed MS<sup>3</sup> was studied by preparing sets of matrix-matched calibration curves at six different concentration levels (5, 10, 20, 50, 100, and 500 µg/l) in apples, fennel seeds,

and at four different concentration levels (20, 50, 100, and 500 µg/l) in sweet pepper, tomatoes, wheat flour, and sesame seeds. The correlation coefficients ( $r^2$ ) for the residue detection of folpet and captan in all the studied matrices are depicted in Tables 2 and 3. As presented,  $r^2$  was higher than 0.997 in all the studied matrices, indicating the high linearity in the studied concentration range.

Ion suppression is generally obtained in LC-QqQ analyses, especially when using electrospray ionization mode

**Fig. 2** Folpet (0.01 mg/kg) analysis using MRM<sup>3</sup> (A1) and MRM (A2) methods and captan analysis (0.01 mg/kg) using MRM<sup>3</sup> (B1) and MRM (B2) methods, in apple

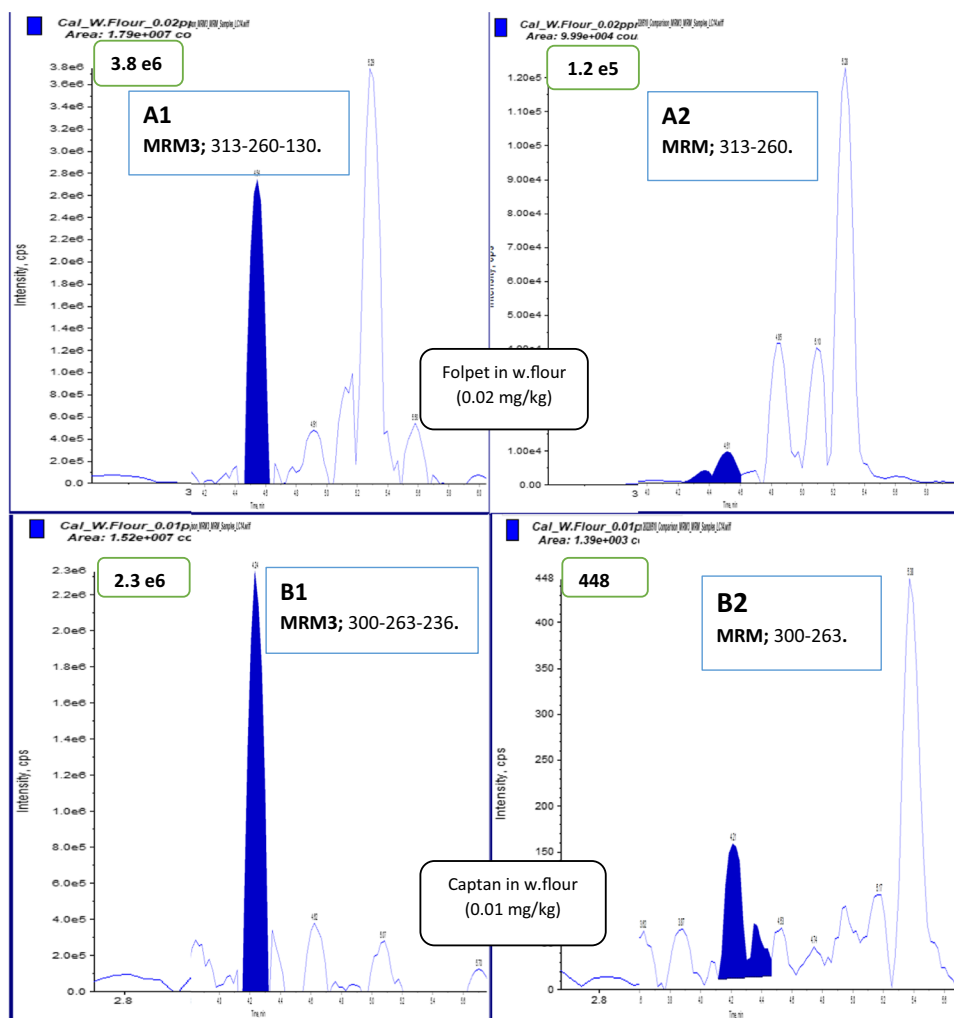


[30]. Therefore, high matrix effects were also observed when using LC-QqQIT analyses of pepper, tomatoes, and wheat flour, as shown in Table 3. These results confirm our suggestion that these selected matrices are complex and challenging commodities for the residue detection of folpet and captan. Even so, LC-QqQIT enables a higher signal-to-noise analysis by minimizing the effect of the co-extracted matrix components. LOQs of folpet and captan in wheat flour and tomatoes are equal to 0.05 mg/kg, which is much lower than their corresponding MRLs [31]. However, a further improvement may be required for sweet pepper samples to achieve a lower LOQ, since its EU-MRL value is 0.03 mg/kg.

## Real sample analyses

The developed LC-QqQIT method was successfully applied for the residue analysis of folpet and captan in 180 real samples, collected from the Egyptian market. As shown in Table 4, apple samples were the most contaminated samples by captan and folpet, respectively, followed by sweet pepper and tomato samples. The highest concentrations of folpet and captan (0.970 and 1.57 mg/kg, respectively) were found in sweet pepper. The presence of folpet and captan above their EU MRLs in some samples reveals the irrational use of these fungicides by some farmers, represents a serious

**Fig. 3** Folpet (0.02 mg/kg) analysis using MRM<sup>3</sup> (A1) and MRM (A2) methods and captan analysis (0.01 mg/kg) using MRM3 (B1) and MRM (B2) methods, in wheat flour



concern, due to their health risk to consumers, and hinders the exportation opportunities.

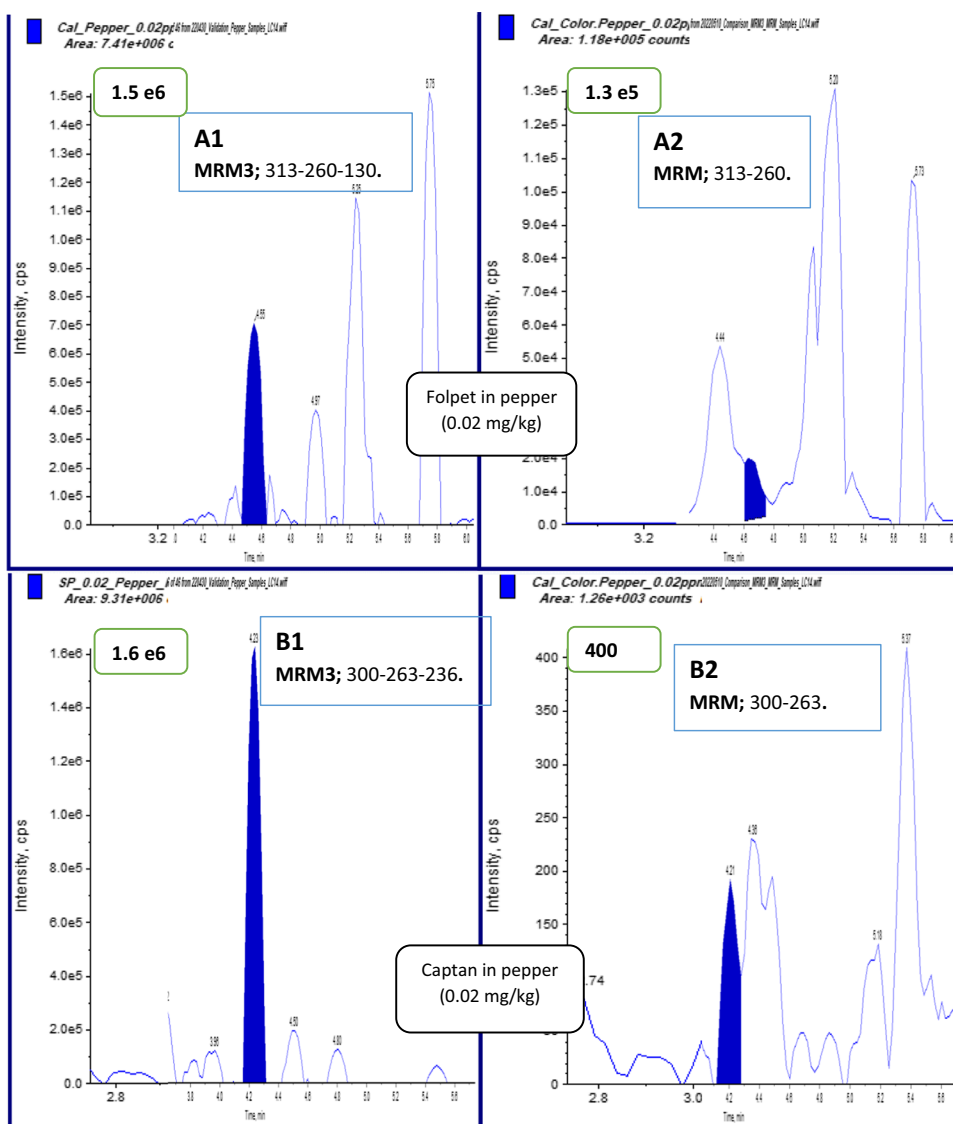
Finally, this survey reflects the efficiency of using the currently developed MS<sup>3</sup> method in the detection of folpet and captan at low residue concentrations in foods, especially for specific consumers like infants and pregnant women, compared to MRM.

## Conclusion

In this study, a rapid and accurate LC-QqQIT (MS<sup>3</sup>) method was optimized for the residue analysis of folpet and captan at lower residue concentrations in foods. The developed method also applies a simple extraction protocol using acidified acetonitrile to most of the studied food samples, except sesame seeds which were extracted using

an acidified acetonitrile/acetone mixture. The developed MS<sup>3</sup> enables the accurate residue detection of folpet and captan even with the sample preparation approach that does not employ a further sample cleaning by a dispersive solid phase extraction (dSPE). The optimized MS<sup>3</sup> method was validated in apples, sesame seeds, sweet pepper, tomatoes, fennel seeds, and wheat flour at low concentration levels ranging from 0.01 to 0.25 mg/kg. It was elucidated that known MRM testing methods cannot be used for the residue analysis of folpet and captan at lower concentrations, compared to the developed MS<sup>3</sup> method. Consequently, the developed MS<sup>3</sup> method enables further safety protections in foods that may be used for infants and pregnant women. Analysis of real samples showed that apple samples were the most contaminated commodities by captan, while the highest detected concentration of folpet and captan was found in sweet pepper.

**Fig. 4** Folpet (0.02 mg/kg) analysis using MRM<sup>3</sup> (A1) and MRM (A2) methods and captan analysis (0.02 mg/kg) using MRM3 (B1) and MRM (B2) methods, in pepper



**Table 2** Recovery (Rec) and relative standard deviation (RSD) with the limit of quantitation (LOQ), matrix effect (ME %), and correlation coefficients ( $R^2$ ) for the residue analysis of folpet and captan in apple

using the developed method on the same day (intraprecision) and in five replicate days (interprecision)

Apple	Intra						Inter		ME %	$r^2$
	0.01 mg/kg		0.02 mg/kg		0.05 mg/kg		0.02 mg/kg			
	Rec	RSD	Rec	RSD	Rec	RSD	Rec	RSD		
Folpet	89	17	109	11	98	9	105	7	-19	0.9999
Captan	83	15	101	7	111	3	97	7	-17	0.9999



**Table 3** Recovery (Rec) and relative standard deviation (RSD) for the residue analysis of folpet and captan in different commodities at 0.01, 0.03, 0.05, and 0.25 mg/kg ( $n=6$ ) using the developed method on the same day (intraprecision), values of the limit of quantitation (LOQ), matrix effect (ME%), and correlation coefficients ( $r^2$ )

Commodity	Concentration (mg/kg)	Rec	RSD	Rec	RSD	ME %	$r^2$
Tomato	0.01 mg/kg			0.05 mg/kg			
	Rec		RSD	Rec	RSD		
Folpet	88	19		112	11	-43	0.9975
Captan	99	19		106	10	-51	0.9975
Sweet pepper	0.03 mg/kg			0.05 mg/kg			
	Rec		RSD	Rec	RSD		
Folpet	104	19		108	6	0.1	0.9993
Captan	91	17		87	18	-34	0.9999
Wheat flour	0.05 mg/kg			0.25 mg/kg			
	Rec		RSD	Rec	RSD		
Folpet	88	18		109	15	-66	0.9994
Captan	113	16		97	5	-53	0.9995
Sesame	0.05 mg/kg			0.25 mg/kg			
	Rec		RSD	Rec	RSD		
Folpet	95	15		118	7	-36	0.9979
Captan	105	19		95	7	-31	0.9997
Fennel	0.05 mg/kg			0.25 mg/kg			
	Rec		RSD	Rec	RSD		
Folpet	102	9		90	11	-33	0.9996
Captan	102	16		102	12	4	0.9996

**Table 4** Levels and the detection frequency of folpet and captan residue in 180 collected real samples (apple, tomato, sweet pepper, wheat flour, sesame seeds, and fennel seeds) were analyzed using the developed method and the EU-published MRL of EU for folpet and captan for these commodities

Commodity	Folpet			MRL-EU (mg/kg)	Captan			
	Range (mg/kg)		Freq detect, %		Range (mg/kg)		Freq detect, %	MRL-EU (mg/kg)
	Min	Max			Min	Max		
Apple	< 0.01*	0.20	17	0.3	< 0.01*	0.52	90	10
Tomato	< 0.01*	0.26	20	5	< 0.01*	0.44	17	1
Sweet pepper	< 0.03*	0.97	20	0.03	< 0.03*	1.57	20	0.03
Wheat flour	0.0933	0.14	7	0.4	< 0.05*	0.35	13	0.07
Sesame seeds	< 0.05*	0.27	13	0.07	< 0.05*	0.20	13	0.07
Fennel seeds	0.028	0.26	10	0.1	< 0.05*	0.32	17	0.1

\*A sample contaminated by a concentration lower than the LOQ

Even though, further studies are still required concerning the usage of the scheduled MS<sup>3</sup> (which is not present in the currently used instrument) to enable the simultaneous multi-detection of a higher number of compounds at the same analysis time. Finally, this new MS<sup>3</sup> testing approach not only assesses the accurate analysis of folpet and captan at low concentrations even in complex food matrices but also resembles a building block for developing similar MS<sup>3</sup> analysis approaches for the residue analyses of other troublesome smaller molecules.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00216-023-04667-x>.

**Acknowledgements** The authors express sincere regard for the technical support from Alexandre Paccou, previous Senior Manager, Service & Support at SCIEEX company, for his valuable and useful technical

support. The authors also thank all staff members at QCAP laboratory, especially Dr. Alla Fathy, for their support during this study.

**Author contribution** Abd-Allah A. El-Sheikh: methodology; Sherif M. Taha: conceptualization, supervision, and writing; Osama H. Elhamalawy: supervision and reviewing; Fawzy I. Eissa: supervision and reviewing.

## Declarations

**Conflict of interest** The authors declare no competing interests.

## References

1. FAO STAT. Food and agriculture organization (FAO) of the United Nations. 2022. <https://www.fao.org/food-agriculture-statistics/en/>.

2. Ge H, Chen L, Su Y, Jin C, Ge RS. Effects of folpet, captan, and captafol on human aromatase in JEG-3 cells. *Pharmacology*. 2018;102:81–7. <https://doi.org/10.1159/000484171>.
3. FAO. Folpet, extensive information. 1998. [http://www.fao.org/fileadmin/templates/agphome/docume%0Ants/Pests\\_Pesticides/JMPR/Evaluation2017/CAPTAN\\_\\_007\\_.pdf](http://www.fao.org/fileadmin/templates/agphome/docume%0Ants/Pests_Pesticides/JMPR/Evaluation2017/CAPTAN__007_.pdf).
4. FAO. Captan, Extensive information. 2017. [https://www.fao.org/fileadmin/templates/agphome/documents/Pests\\_Pesticides/JMPR/Evaluation2017/CAPTAN\\_\\_007\\_.pdf](https://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation2017/CAPTAN__007_.pdf).
5. Berthet A, Bouchard M, Schüpfer P, Vernez D, Danuser B, Huynh CK. Liquid chromatography–tandem mass spectrometry (LC/APCI-MS/MS) methods for the quantification of captan and folpet phthalimide metabolites in human plasma and urine. *Anal Bioanal Chem*. 2011;399:2243–55. <https://doi.org/10.1007/s00216-010-4601-z>.
6. Gordon EB. Captan and Folpet. Third Edit: Elsevier Inc; 2004.
7. Bellisai G, Bernasconi G, Brancato A, Cabrera LC, Castellan I, Ferreira L, Giner G, Greco L, Jarrah S, Leuschner R, Magrans JO, Miron I, Nave S, Pedersen R, Reich H, Robinson T, Ruocco S, Santos M, Scarlato AP, Theobald A (2022) Modification of the existing maximum residue level for folpet in lettuces. 20:1–24. <https://doi.org/10.2903/j.efsa.2022.7309>.
8. Organtini K, Leonard S, Hird S, Mccall E, Cleland G. A new strategy for the determination of captan and folpet in food matrices a. *Waters Appl Notes*. 2017. [https://www.waters.com/webassets/cms/library/docs/2017nacrw\\_organtini\\_unispray.pdf](https://www.waters.com/webassets/cms/library/docs/2017nacrw_organtini_unispray.pdf).
9. Huertas-Pérez JF, Ernest M, Varela J, Badoud F. Quantification of folpet and phthalimide in food by gas chromatography and mass spectrometry: overcoming potential analytical artefacts. *Food Chem*. 2018;260:213–20. <https://doi.org/10.1016/j.foodchem.2018.04.002>.
10. Geis-Asteggiate L, Lehotay SJ, Heinzen H. Effects of temperature and purity of magnesium sulfate during extraction of pesticide residues using the QuEChERS method. *J AOAC Int*. 2012;95:1311–8. [https://doi.org/10.5740/jaoacint.SGE\\_Geis-Asteggiate](https://doi.org/10.5740/jaoacint.SGE_Geis-Asteggiate).
11. EURL-SRM. Analysis of captan, folpet and their respective metabolites phthalimide and tetrahydrophthalimide via LC-MS/MS either directly or following hydrolysis. In: *Anal Obs Rep*. 2019. [http://www.eurl-pesticides.eu/library/docs/srm/EurlSrm\\_Observation\\_Captan\\_Folpet\\_LC-V1.pdf](http://www.eurl-pesticides.eu/library/docs/srm/EurlSrm_Observation_Captan_Folpet_LC-V1.pdf).
12. Schenck FJ, Lehotay SJ. Does further clean-up reduce the matrix enhancement effect in gas chromatographic analysis of pesticide residues in food? *J Chromatogr A*. 2000;868:51–61. [https://doi.org/10.1016/S0021-9673\(99\)01137-1](https://doi.org/10.1016/S0021-9673(99)01137-1).
13. European Commission Regulation. Amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for boscalid, clothianidin, thiamethoxam, folpet and tolclofos-methyl in or on certain products. *Off J Eur Union*. 2018;2016:48–119.
14. European Commission Regulation. Amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for captan, propiconazole and spiroxamine in or on certain products. *Off J Eur Union*. 2018;2016:48–119.
15. Abo-Gaida AAH, Shendy AH, Taha SM, Mahmoud HA, Attallah ER, Fernandez-Alba AR. Fennel-seeds extract as an analyte protectant for the GC-MS/MS residue analysis of 182 pesticide in strawberries: comparing the manual mixing and sandwich injection. *J Chromatogr Open*. 2022;2:100056. <https://doi.org/10.1016/j.jcoa.2022.100056>.
16. Uclés S, Hakme E, Ferrer C, Fernández-Alba AR. Analysis of thermally labile pesticides by on-column injection gas chromatography in fruit and vegetables. *Anal Bioanal Chem*. 2018;410:6861–71. <https://doi.org/10.1007/s00216-018-1286-1>.
17. Cutillas V, Jesús F, Ferrer C, Fernández-Alba AR (2021) Overcoming difficulties in the evaluation of captan and folpet residues by supercritical fluid chromatography coupled to mass spectrometry. *Talanta* 223. <https://doi.org/10.1016/j.talanta.2020.121714>.
18. Oulkar DP, Shinde R, Khan Z, Organtini K, Leonard S, Banerjee K. Improved analysis of captan, tetrahydrophthalimide, captafol, folpet, phthalimide, and iprodione in fruits and vegetables by liquid chromatography tandem mass spectrometry. *Food Chem*. 2019;301:125216. <https://doi.org/10.1016/j.foodchem.2019.125216>.
19. Shinde R, Shiragave P, Lakade A, Thorat P, Banerjee K. Multi-residue analysis of captan, captafol, folpet, and iprodione in cereals using liquid chromatography with tandem mass spectrometry. *Food Addit Contam - Part A Chem Anal Control Expo Risk Assess*. 2019;36:1688–95. <https://doi.org/10.1080/19440049.2019.1662953>.
20. Oppermann U, Moreau S, Toinon D, Europa S, Corporation GS. Analysis of captan, folpet and their derivatives in food with APCI-LCMS-8060. In: SHIMADZU Appl Notes. 2007. [https://www.shimadzu.eu/sites/shimadzu.seg/files/SEG-images/Events/PDFs/Analysis\\_of\\_Captan\\_Folpet\\_and\\_their\\_derivatives\\_in\\_food\\_with\\_APCI-LCMS-8060.pdf](https://www.shimadzu.eu/sites/shimadzu.seg/files/SEG-images/Events/PDFs/Analysis_of_Captan_Folpet_and_their_derivatives_in_food_with_APCI-LCMS-8060.pdf).
21. Barreda M, López FJ, Villarroya M, Beltran J, García-BAUDÍN JM, Hernández F. Residue determination of captan and folpet in vegetable samples by gas chromatography/negative chemical ionization-mass spectrometry. *J AOAC Int*. 2006;89:1080–7. <https://doi.org/10.1093/jaoac/89.4.1080>.
22. Zang X, Wang J, Wang O, Wang M, Ma J, Xi G, Wang Z. Analysis of captan, folpet, and captafol in apples by dispersive liquid-liquid microextraction combined with gas chromatography. *Anal Bioanal Chem*. 2008;392:749–54. <https://doi.org/10.1007/s00216-008-2296-1>.
23. King R, Fernandez-metzler C. The use of Qtrap technology in drug metabolism. 2006;7:541–5. <https://doi.org/10.2174/13892006777697936>.
24. Pailleux F, Beaudry F. Evaluation of multiple reaction monitoring cubed for the analysis of tachykinin related peptides in rat spinal cord using a hybrid triple quadrupole-linear ion trap mass spectrometer. *J Chromatogr B*. 2014;947–948:164–7. <https://doi.org/10.1016/j.jchromb.2013.12.025>.
25. Lakowski TM, Szeitz A, Pak ML, Thomas D, Vhuiyan MI, Kotthaus J, Clement B, Frankel A. MS 3 fragmentation patterns of monomethylarginine species and the quantification of all methylarginine species in yeast using MRM 3. *J Proteomics*. 2013;80:43–54. <https://doi.org/10.1016/j.jprot.2013.01.003>.
26. Pihlström T, Fernández-Alba AR, Ferrer Amate C, Erecius Poulsen M, Lippold R, Carrasco Cabrera L, Pelosi P, Valverde A, Mol H, Jezussek M, Malato O, Štěpán R. Analytical quality control and method validation procedures for pesticide residues analysis in food and feed SANTE 11312/2021. 2022. [https://www.eurl-pesticides.eu/userfiles/file/EurlALL/SANTE\\_11312\\_2021.pdf](https://www.eurl-pesticides.eu/userfiles/file/EurlALL/SANTE_11312_2021.pdf). Accessed 22 Mar 2022.
27. Taha SM. A rapid sensitive and selective GC-MS/Ms method for multi residue analysis of a large number of pesticides in chamomile. *Egypt J Chem*. 2021;64:605–22. <https://doi.org/10.21608/EJCHEM.2020.51066.3046>.
28. Taha S, Al-Kharosi A, Al-Sabari W, AL-shidhani F, Alfarsi S (2020) A simple method for simultaneous determination of commonly used artificial food colors and preservatives in soda, jam, and yogurt by HPLC-PDA. *Brazilian J Anal Chem* 7. <https://doi.org/10.30744/brjac.2179-3425.AR-23-2019>.
29. Gross JH. Textbook, mass spectrometry, 3rd ed. Springer Int Publ AG 1040:1–4. 2017. <https://doi.org/10.1007/978-3-319-54398-7>.
30. Monteiro SH, Lehotay SJ, Sapozhnikova Y, Ninga E, Lightfield AR, Ninga E, Lightfield AR. High-throughput mega-method for the analysis of pesticides, veterinary drugs, and environmental contaminants by ultra-high-performance liquid chromatography-tandem mass spectrometry and robotic mini-solid-phase extraction cleanup + low-pres. 2020.

31. European Commission. Directorate-general, EU pesticides database, pesticide residues, and the MRLs. In: Regul. No 1107/2009. 2021. <https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/mrls>.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.