Chapter 8

Determination of Triazole Fungicides in Fruits and Vegetables by Liquid Chromatography-Mass Spectrometry (LC/MS)

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Additional information is available at the end of the chapter

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1. Introduction

Triazole pesticides derivatives represent the most important category of fungicides that have excellent protective, curative and eradicant power towards a wide spectrum of crop diseases [1]. The fungicide group, demethylation inhibitors (DMI), which contain the triazole fungicides, was introduced in the mid-1970s. These fungicides are highly effective against many different fungal diseases, especially powdery mildews, rusts, and many leaf-spotting fungi. [2].

The number of pesticides registered for use increases every year and many pesticides that have been banned for health reasons are also still being used illegally. And introduction of new pesticides in the field of residue analysis also cause the laboratories involved in the analysis to face more challenging task. This leads to the development of many multi-residue methods by various researchers [3-7].

In the past, pesticides and their degradation products, which are generally thermolabile, non-volatile and exhibit medium to high polarity have been analysed using GC with specific detectors such as ECD, NPD and FPD [8-12]. Due to the drawbacks of the separation techniques such as sensitivity, insufficient number of analytes that can be analysed and the need for confirmation either with different column polarity or detectors, GC/MS has become the primary approach to analyse all classes of GC-amenable pesticides [3, 13-14]. Later, HPLC combined with a diode array UV detector was established as a complementary technique to GC to analyse pesticides and their degradation products [15]. However it is not sufficient to use only the UV spectrum for identification of the analytes. Robust atmospheric pressure ionization (API) ion source designs, which consist of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) were developed and very powerful

and reliable LC/MS instruments have been introduced commercially. The atmospheric pressure interfaces has been used to broaden the range of analytes to be analysed by liquid chromatography coupled with mass spectrometry [16-17].

Solvents such as acetone, ethyl acetate and acetonitrile may be used for extraction. However, acetonitrile is the recommended solvent and is being used widely for QuEChERS method because when salts are added, it separates more easily from water than acetone. Ethyl acetate has the advantage of partial miscibility with water but it co-extracts with lipids and waxes giving lower recoveries for acid-base pesticides [6]. A study by Lehotay S.J et al in 2010 [18] showed that results using acetate-buffered MeCN gave more accurate (true and precise) results for all analytes in LC-MS/MS than EtOAc. On the contrary EtOAc is a better solvent for GC rather than MeCN as demonstrated by the slightly more consistent recoveries and reproducibility overall in GC-MS using EtOAc.

2. Materials and methods

2.1. Equipment

i. LC/MS instrument

The chromatographic system used to analyse the extract is a Waters Alliance Separations Module 2695 equipped with a quaternary solvent delivery system, autosampler and column heater. A Waters ZQ 4000 single quadrupole Mass Spectrometer was used.

ii. Chopper and Vortex mixer

Robot Coupe R5 V.V (Jackson, MS) and OMNI mixer homogenizer (OMNI International, USA) were used to cut the fruit and vegetable samples into smaller pieces. Genie II vortex mixer was used to swirl the tubes.

iii. Centrifuge

Sorvall Legend RT Plus / Thermo Scientific were used for the centrifugation.

iv. Balance

A Shimadzu top-loading balance Libror AEG-220 was used to weigh the chopped samples and solid reagents and a Shimadzu analytical balance Libror EB-3200 HU was used in the preparation of stock standard solutions.

v. Vials and tubes

For the extraction step, 50mL centrifuge tubes were employed. 15mL graduated centrifuge tubes were used for dispersive SPE in the method.

vi. Solvent Evaporator

Zymark nitrogen evaporator Turbovap LP was used to concentrate the extracts and to facilitate solvent exchange when necessary.

2.2. Chemicals and reagents

The fungicides: cyproconazole, difenoconazole, fenbuconazole, hexaconazole, myclobutanil, propiconazole, tebuconazole, triadimefon and triadimenol were purchased from Pestanal, Riedel-de Haen (Seelze, Germany) with purity ranging from 95-100%. Acetonitrile, methanol, ethyl acetate of HPLC grade and residue analysis grade were obtained from Labscan and Merck (Darmstadt, Germany). Formic acid which was added to the mobile-phase acetonitrile was purchased from Fluka.

Salts used for the dispersive clean-up were anhydrous magnesium sulfate and sodium acetate which were obtained from Merck and Mallinckrodt. The SPE sorbent used was Bondesil PSA, 40μm from Varian. Deionized water (<8cm MΩ resistivity) was obtained from the Milli-Q Advantage A10 Pure Water System (Millipore, Bedford, MA, USA). All solvents were filtered using a 0.45μm nylon membrane filter from Whatman (Maidstone, England).

2.2.1. Stock and working solutions

Stock solutions of 1000 μg/ml were prepared in methanol by dissolving approximately 0.020g of the individual standards in 20mL of methanol and stored at 4°C in a reagent bottle. Intermediate standard solution mixtures of 50 and 10 μg/ml were prepared in methanol and standard working solutions at various concentrations were prepared daily by appropriate dilution of the stock solution or the intermediate standard solution in methanol.

2.3. Methods

2.3.1. Extraction and clean-up

The extraction method used was based on QuEChERS method [6] and modified by Aysal et al., 2007 [7]. The samples were chopped into smaller pieces and homogenised using a food processor. 30g of the homogenised sample was placed in a 250ml borosilicate bottle and extracted with 60ml of ethyl acetate, 30g of anhydrous sodium sulfate and 5g of sodium hydrogen carbonate. 10 ml of the extract was centrifuged at 2500 rpm for 2 min followed by clean-up with PSA sorbent and anhydrous magnesium sulfate. After clean-up, 5 mL of the extract was reduced to almost dryness under a stream of nitrogen and was redissolved in methanol.

2.3.2. Recovery studies

Four types of fruits and vegetables namely carrot, cabbage, tomato and orange were used for the recovery studies which represent root and tuber vegetables, brassica leafy vegetables, fruiting vegetables and citrus fruits according to the CODEX classification of commodities.

The samples for recovery determination were prepared by spiking with the standard solution. Each sample was fortified with nine triazole standards at 0.05, 0.5 and 1.0 μg/ml and five replicates at these fortification levels for each matrix. The fortified samples were

allowed to stand for 30 min before extraction to allow the spiked solutions to penetrate the samples and attain the fungicide distribution in the samples.

2.3.3. Calibration

Quantification of triazoles were performed and compared by using calibration standards involving both matrix-matching by adding standards to blank extracts and non-matrix matching (standards in solutions) based on a calibration curve. For matrix matching, blank extracts were fortified with the pesticide working standard after dispersive clean-up. The calibration solutions were prepared daily at 7 levels of concentrations ranging from 0.05 to 2.0 μg/ml. The LOD's and LOQ's were calculated by multiplying the standard deviation of the calculated amount for each triazole by 3 and 10 respectively.

3. Results and discussion

3.1. High Performance liquid chromatography-mass spectrometry

3.1.1. HPLC

A C18 reversed phase column (4.6mm x 75mm, 3.5 um particle size) was used in this study to generate less back pressure as it allows more flexibility to adjust the flow-rate. A short column was also used to obtain shorter separation times that produce narrower peaks because there is less time for diffusive broadening. The small particle size used helps to generate more pressure and generally give higher separation efficiencies. Smaller particle size column is necessary to maintain resolution in the short column used. The HPLC column had been run at different flow rates; 0.8 mL/min, 1 mL/min, 1.2 mL/min and 1.4 mL/min during optimization and it was found that it gives better resolution at a flow rate of 1.2 mL/min A common operating temperature is 40°C as higher temperature is better in producing sharp peaks and earlier elution [19]. For this study, the effects of column temperature were also evaluated at various temperatures; 20°C, 25°C, 30°C, 35°C and 40°C. Figure 1 showed that 25°C column temperature found to give better separation after running triazole standard mixture.

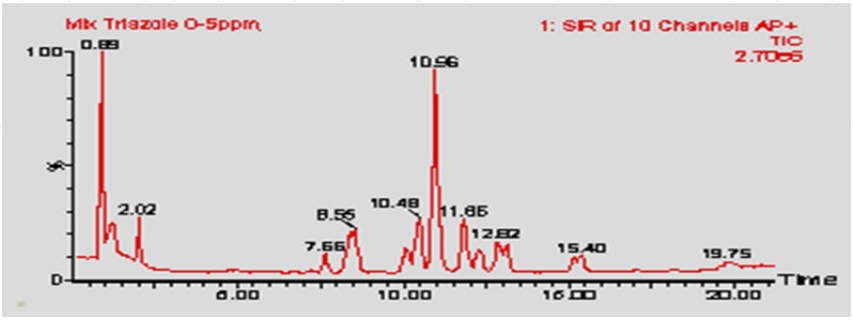

Figure 1. Acetonitrile/ H_2O mobile phase, column temperature 25°C

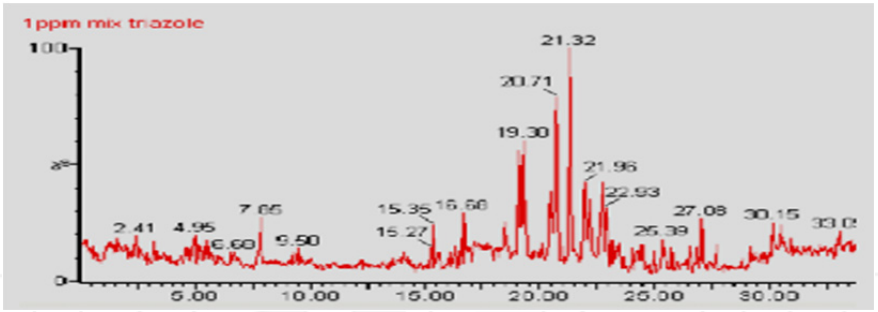

Figure 2. Acetonitrile/ H_2O mobile phase, column temperature 35°C

In this work, water and acetonitrile with 0.1% acetic acid were used for all liquid chromatographic separations. No buffers were used. Two types of additives have been added to the mobile phase that is acetic acid and formic acid during optimization and it was found that by adding 0.1% of acetic acid gives better resolution than formic acid. The results are also more stable. The additive was added to improve the chromatographic shape and to provide a source of protons in the reversed phase and to enhance and control the formation of ions. A study on water:methanol with both 0.1% acetic acid and formic acid was also done but it did not give good resolution and the results are not reproducible.

The reversed phase solvents are installed on the channels A and C. Channel A is the aqueous solvent (water) and channel C is the organic solvent (acetonitrile). Silica dissolves at high pH, therefore it is not recommended to use solvents that exceed pH7. The pH for acetonitrile was in the range of pH 2.5 – pH 3.5.

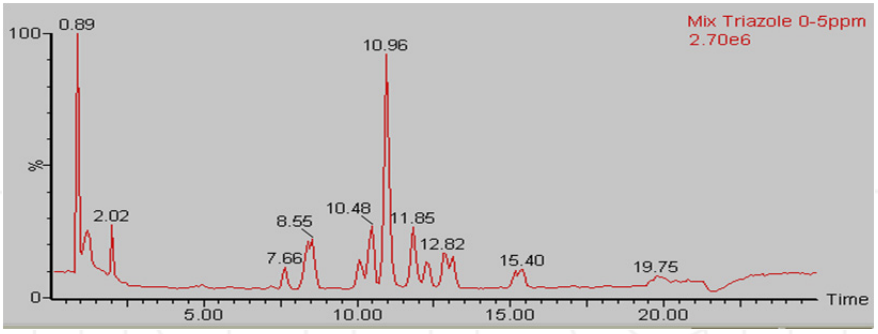

Figure 3. Chromatogram of triazole standards mixture at 0.5 µg/mL

3.2. Mass spectrometry

Prior to triazole analysis, the chromatographic parameters including the heated nebuliser parameters were optimized. LCMS infusion was carried out to examine the ionization and fragmentation patterns of the analytes. The APCI source was used in the positive ion mode. A full scan was used for the MS optimization and a selected ion monitoring (SIM) was used for the monitoring of the selected ion. Table 1 showed the triazoles and quantitation ion.

Analyte	t_R, min	Quantitation ion, m/z
Triadimenol	7.66	296.1
Cyproconazole	8.55	292.1
Myclobutanil	10.08	289.1
Triadimefon	10.48	294.1
Tebuconazole	10.96	308.1
Hexaconazole	11.85	314.1
Fenbuconazole	12.25	337.1
Propiconazole	12.82	342.1
Difenoconazole	13.14	406.2

t_R = Retention time

Table 1. List of Triazole, their retention time and Quantitation ions

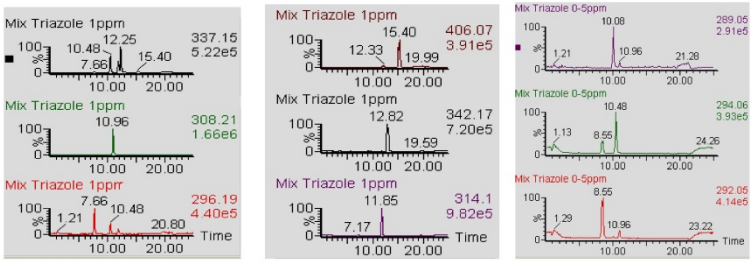

Figure 4. Mass spectra of triazoles; Fenbuconazole (337.1), Difenoconazole (406.1), Myclobutanil (289.1), Tebuconazole (308.1), Propiconazole (342.1), Triadimefon (294.1), Triadimenol (296.1), Hexaconazole (314.1) and Cyproconazole (292.1).

3.2.1. Mass spectrometer tuning

Before the chromatographic method was established, the mass spectrometer was tuned to optimize the conditions of parameter for both the formation and detection of ions during an analysis. It is also done to increase the sensitivity and to optimize the mass peak resolution for the application. Optimization of both the ionization process and ion transportation in the mass spectrometer is important to achieve high sensitivity and selectivity and low detection limits in liquid chromatography / atmospheric pressure chemical ionization spectrometry (LC/APCI-MS) analysis. The optimization was done by changing one-variable-at-a time while the others are kept constant.

The mass spectrometer tuning was done using two methods; by infusing a sample with the syringe pump and also from the syringe pump into the LC flow line. This is to see the effect of mobile phase flow rate and composition on signal intensity and to allow optimization of the source parameters without making numerous injections in order to achieve parameters giving the highest sensitivity. Infusion experiments were carried out to examine the ionization and fragmentation patterns of the analytes. The instrument parameter; corona voltage, cone voltage, desolvation flow and temperature, cone flow and mass resolution

were optimized to provide the best possible sensitivity by infusion. Corona voltage, was studied in the range from 3.5 V to 5 V and cone voltage studied in the range from 25 V to 35 V.

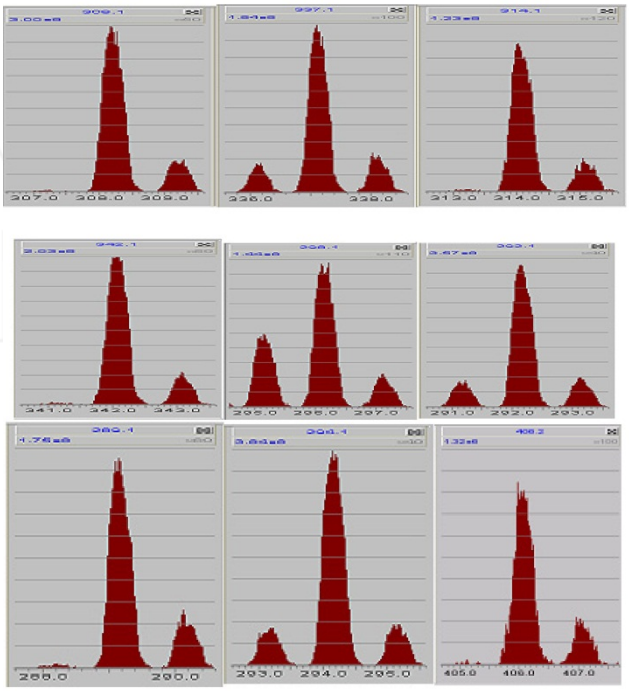

Figure 5. Mass of triazoles after tuning; (308.1), Fenbuconazole (337.1), Hexaconazole (314.1), Propiconazole (342.1), Triadimenol (296.1), Cyproconazole (292.1), Myclobutanil (289.1), Triadimefon (294.1) and Difenoconazole (406.2).

3.3. Sample preparation and extraction

3.3.1. Sample preparation

The analysis is performed on a subsample of the laboratory sample, after appropriate comminution. This is to ensure the sub-sample is representative of the original laboratory samples.

In this study, 1-2 kg laboratory samples; cabbage, carrot, tomato and orange were used as representative samples. The laboratory samples were processed in a large chopper (Robot Coupe R5 V.V) and were blended to a consistent texture. Then 200g of the comminuted samples was transferred to another container and homogenized with the OMNI mixer homogenizer until homogeneous. This step is taken so that the 30 g samples taken for extraction are highly representative of the initial sample. Well comminuted samples can improve the shaking based extraction and less time is spent on the overall homogenization

of the large initial laboratory samples [20]. An extremely homogeneous sample also maximizes surface area and ensures better extraction efficiencies.

30 g sub-sample was used by Aysal et al., 2007 [7] based on a study by Maestroni et al. 2000 [21-22] that showed results produced using the same chopper in the same laboratory gave representative results within generally ≤ 8% relative error of the mean concentration of the original sample.

3.3.2. Sample extraction

In contrast with acetone and acetonitrile-based methods, in which SPE is commonly employed, it has been reported only occasionally or no clean-up for ethyl acetate-based methods; however in this study dispersive-SPE clean-up was performed. Mol, H.G.J et al., 2007 [23] showed that laborious steps in multi residue analysis can be replaced by more efficient alternatives including the clean-up process. Solid-phase extraction previously used in the clean-up procedure which involves less dilution and is less laborious can be replaced by dispersive SPE, as described by Anastassiades et al., 20 [6].

SPE clean up used plastic cartridges containing various amounts of sorbent material and the procedures involve conditioning, sample transfer, elution, and evaporative re-concentration [23]. For this study, in the dispersive-SPE clean-up, 0.25 g primary secondary amine (PSA) and 1.5 g of anhydrous magnesium sulfate ($MgSO_4$) were added to a 10 mL aliquot of the sample extract and the mixture is mixed using a vortex mixer to evenly distribute the SPE material and facilitate the clean up process. The sorbent is then separated by centrifugation and the supernatant is ready for analysis. The function of the sorbent is to retain matrix components and not the analytes of interest. In some instances, other sorbents or mixed sorbents can be used depending on the samples and analytes.

A difficulty that was encountered by using ethyl acetate is that some of the most polar pesticides do not readily partition into ethyl acetate. It co-extracts with lipids and waxes, giving lower recoveries for the acid-base pesticides, it is sufficiently polar to penetrate into the cells of the matrix and it dissolves a great number of polar pesticides and their metabolites. On the other hand, ethyl acetate is partially miscible with water and the advantage is that it makes the addition of other non-polar solvents to separate water from the extract unnecessary. To increase the recoveries of polar compounds, large amounts of sodium sulfate (Na_2SO_4) are usually added in the procedures using ethyl acetate to bind the water. Polar co-solvents, such as methanol and ethanol, have been used to increase the polarity of the organic phase [23-26].

Different types of samples have different pH values that can affect the recoveries of pH-susceptible pesticides and their stability in the extracts. Therefore the pH of the extracts for some samples must be controlled [6, 20, 27]. Most pesticides are more stable at lower pH. Problematic pesticides that are strongly protonated at low pH the extracts must be buffered in the range of pH 2-7 [28]. The pH at which the extraction is performed can also influence the co-extraction of matrix compounds and pesticide stability. The pH of the samples

extracted in this study was between pH 2.5 – pH 4.0. Sodium hydrogen carbonate ($NaHCO_3$) was added in the method to give a consistent pH during extraction independent of the initial sample pH.

Aysal P. et al., 2007 [7] mixed the sample 1:1 (w:w) with anhydrous sodium sulfate (Na_2SO_4) and used a 2:1 (v:w) ethyl acetate : sample ratio because it had been evaluated previously to achieve high recoveries. It resulted in good extraction efficiency and is practical with regard to achieving phase separation and avoidance of emulsions. [23-25].

The two conditions most relevant to extraction efficiency are the sample-to-solvent ratio and the addition of salt, which in ethyl acetate-based multi-residue methods has always been sodium sulfate. A study done by Mol, H.G.J et al. in 2003 [23] showed that the addition of salt improves the extraction efficiency for polar pesticides.

3.3.3. Dispersive-SPE clean-up

The purpose of salt addition is to induce phase separation. The salting-out effect also influences analyte partition, which is dependent upon the solvent used for extraction. The concentration of salt can influence the percentage of water in the organic phase and can adjust its "polarity". In the QuEChERS method, acetonitrile alone is often sufficient to perform excellent extraction efficiency without the need to add non-polar co-solvents that dilute the extract and make the extracts too non-polar. By using deuterated solvents in the nuclear magnetic resonance studies, Anastassiades and colleagues [6] investigated the effect of various salt additions on the recovery and other extraction parameters. They studied the effect of polarity differences between the two immiscible layers. The use of magnesium sulfate as a drying salt to reduce the water phase helped to improve recoveries by promoting partitioning of the pesticides into the organic layer. To bind a significant fraction of water, the amount of magnesium sulfate exceeded the saturation concentration. The supplemental use of sodium chloride helps to control the polarity of the extraction solvents and thus influences the degree of matrix clean up of the QuEChERS method but too much of this salt will reduce the organic layer's ability to partition polar pesticides.

Dispersive solid-phase extraction is similar in some respects to matrix solid-phase dispersion developed by Barker [28-29] but in this instance, the sorbent is added to an aliquot of the extract rather than to the original solid sample as in matrix solid-phase extraction. In dispersive solid-phase extraction, a smaller amount of sorbent is used only because an aliquot of the sample is subjected to the clean up. Compared with SPE, dispersive solid-phase extraction takes less time and uses less labour and lower amounts of solvent without the extra steps such as channeling, analyte or matrix breakthrough, or preconditioning of SPE cartridges. Just as a drying agent is sometimes added to the top of an SPE cartridge, magnesium sulfate is added simultaneously with the SPE sorbent to remove much of the excess water and to improve the analyte partitioning to provide better clean up.

3.4. Quantitative determinations

All samples were quantified using the method of external standards. The linear concentration range was derived from the calibration graphs. Seven-point calibration curves for each compound were found to be linear ranging from 0.05 to 2.0 μg/ml, with 1/x weighting and with correlation coefficients (r^2) of >0.995. SIM traces were integrated for quantitation purposes. The limit of detection (LOD) and limit of quantification (LOQ) were estimated from the computer-generated software using a signal-to noise ratio (S/N) program. The LOD and LOQ were determined based on a signal to noise ratio of 3 and the limit of quantification (LOQ) was based on a signal to noise ratio of 10.

The internal standard was evaluated qualitatively only to confirm the injection of the sample extract. Normalization against the internal standard was not considered feasible because of unpredictable and varying matrix effects for several of the matrices studied in this work. A matrix-matched standard was also prepared by spiking the final extract; a fortification standard was added to the blank sample that had been extracted using the same procedure.

3.5. Pesticide recoveries

Recovery of pesticides from the fortified samples was calculated relative to that from a solvent standard and a matrix-matched standard. The acceptable percentage ranges for recovery (accuracy) and CV (precision) was based on CODEX criterion for method validation.

Recoveries and coefficients of variation of triazoles from fortified orange samples are shown in Table 2 and Fig. 6. Samples were spiked at 0.05mg/kg, 0.5mg/kg and 1mg/kg. The recoveries for these triazoles were from 60% to 145% with CV of 2.3 to 13.1% Most of the compound recoveries give more than 70% and fufills the codex acceptable recovery range. The recovery for Cyproconazole, tebuconazole and propiconazole at 1.0 mg/kg and myclobutanil at 0.05 mg/kg falls outside the acceptable range but the CV is within the acceptable range. Overall average recovery was 95% at all 3 fortification levels and all compounds met the CODEX CV acceptable range.

	Orange		
	1.0 mg kg^1	**0.5 mg kg^{-1}**	**0.05 mg kg^{-1}**
Triadimenol	9.3 (2.3)	59.6 (7)	89.1 (11.4)
Cyproconazole	128.8 (4.9)	88.9 (4.7)	75.2 (4.9)
Myclobutanil	103.0 (3.9)	83.9 (5.5)	144.9 (4.8)
Triadimefon	89.7 (2.7)	65.7 (7.1)	85.9 (3.1)
Tebuconazole	142.4 (5.5)	98.5 (4.0)	100.9 (5.3)
Fenbuconazole	111.4 (3.9)	73.1 (5.9)	91.84 (13.1)
Hexaconazole	117.0 (7)	87.0 (5.4)	74.8 (6.4)
Propiconazole	121.5 (3.2)	75.0 (4.7)	69.4 (9.8)
Difenoconazole	118.7 (4.3)	86.2 (5.9)	87.7 (9)

Table 2. Recovery of Triazoles in orange (n=5)

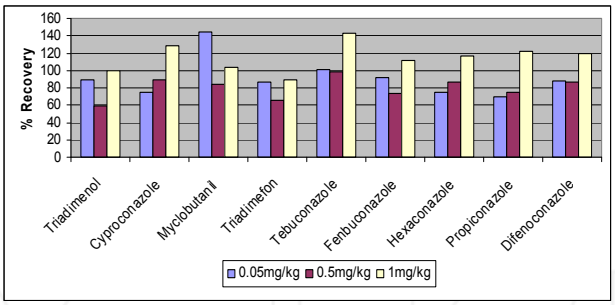

Figure 6. Recoveries fortified in Orange (n=5 at each level).

The recoveries and coefficients of variation of triazoles from fortified cabbage samples are shown in Table 3 and Fig. 7. Samples were spiked at 0.05mg/kg, 0.5mg/kg and 1mg/kg. The recoveries for 9 triazoles were within the acceptable range for 1 mg/kg fortification levels. They ranged between 98-120% (CV of 2.6% to 7.1%). Recoveries obtained at 0.5 mg/kg were in the range of 76-100% with CV of 4.1 to 18.9%). The recoveries for cyproconazole and fenbuconazole at 0.5 mg/kg were in the acceptable range but CV% was out of range. Recoveries for cabbage spiked at 0.05mg/kg were between 53-98%. A lower recovery was obtained for cyproconazole and tebuconazole while fenbuconazole was almost all lost (0.7%) and the CV was also so high (149.9%). The overall average recovery was 91%.

	Cabbage		
	1.0 mg kg^{1}	**0.5 mg kg^{-1}**	**0.05 mg kg^{1}**
Triadimenol	115.8 (2.6)	99.0 (4.1)	88.7 (2.5)
Cyproconazole	98.6 (7.1)	76.0 (16.8)	53.5 (13.0)
Myclobutanil	119.9 (2.7)	98.8 (6.3)	92.1 (4.1)
Triadimefon	112.9 (6.0)	100.3 (5.1)	101.9 (2.3)
Tebuconazole	98.9 (3.4)	77.8 (10.0)	58.9 (6.9)
Fenbuconazole	101.8 (6.1)	76.5 (18.9)	0.7 (149.4)
Hexaconazole	115.6 (2.7)	94.2 (8.3)	71.5 (3.8)
Propiconazole	113.15 (4.3)	96.6 (4.8)	96.4 (3.6)
Difenoconazole	112.4 (5.5)	95.6 (8.1)	98.3 (9.9)

Table 3. Recovery of Triazoles in cabbage (n=5)

The recovery results obtained from spiked tomato at different levels was between 66-144% (see Table 4). The CV for all compounds at different fortification levels were in the range of 2.6 – 11.9% and met the CV acceptable range except for fenbuconazole (16.9%). The recovery for propiconazole at all the 3 concentrations of 0.05 mg/kg and 0.5 mg/kg spiked did not meet the acceptance limit but the CV's met the acceptable limit. The overall average recovery was 105.3%.

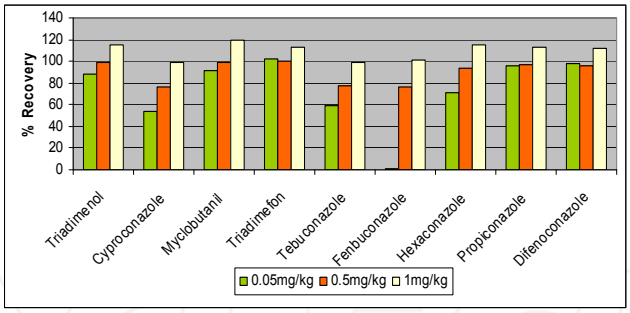

Figure 7. Recoveries fortified in Cabbage (n=5 at each level)

	Tomato		
	1.0 mg kg^{1}	**0.5 mg kg^{-1}**	**0.05 mg kg^{-1}**
Triadimenol	105.7(10.3)	95.7 (8.9)	87.8 (9.1)
Cyproconazole	97.1 (4.2)	88.8 (8.3)	92.6 (11.9)
Myclobutanil	91.9 (8.3)	88.0 (8.6)	66.2 (10.9)
Triadimefon	103.6 (9.1)	96.4 (6.3)	118.9 (10.7)
Tebuconazole	105.6 (8.8)	88.9 (8.9)	115.2 (10.3)
Fenbuconazole	104.0 (2.6)	93.8 (5.8)	103.4 (16.9)
Hexaconazole	108.3 (9.4)	86.6 (10.0)	84.9 (8.0)
Propiconazole	188.0(10.2)	144.7 (4.6)	175.6 (7.1)
Difenoconazole	107.1(15.7)	99.7 (9.7)	105.2 (10.7)

Table 4. Recovery of Triazoles in tomato (n=5)

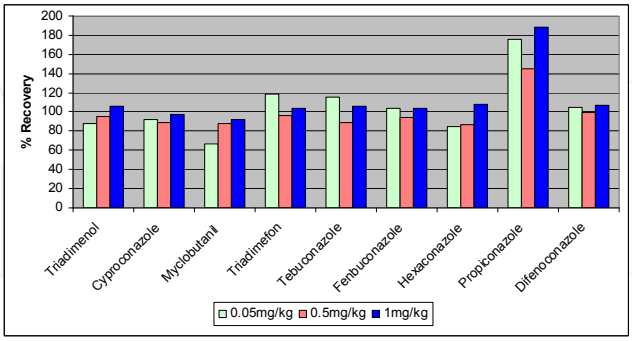

Figure 8. Recoveries fortified in tomato (n=5 at each level)

The recoveries for all spiked triazoles at 3 concentration levels in carrot are shown in Table 5 and Figure 9. As shown in Table 5, the recoveries for carrot spiked at 0.05 mg/kg were quite low for most of the analytes, between 48-111% with CV in the range of 5.4 to 56.6%. The

recovery spiked at 0.5 mg/kg was between 68-85% but difenoconazole recovery was very high at 151%. Contrary to the recovery spiked at 0.05 mg/kg, recoveries spiked at 1 mg/kg were very high (more than 100%) for most compounds between 86-142% and CV for all compounds did not meet the range. The overall average recovery was 90.7% for all fortification levels.

	Carrot		
	1.0 mg kg^{-1}	**0.5 mg kg^{-1}**	**1.0 mg kg^{-1}**
Triadimenol	86.0 (15.2)	69.8 (7.4)	48.3 (6.1)
Cyproconazole	113.0 (14.2)	72.9(11.0)	64.7 (8.6)
Myclobutanil	126.0 (14.4)	75.3 (8.2)	54.7 (5.4)
Triadimefon	121.0 (18.8)	68.6 (8.2)	50.4 (10.8)
Tebuconazole	142.0 (15.5)	75.5 (7.3)	73.9 (46.7)
Fenbuconazole	109.0 (14.3)	74.4 (8.0)	51.0 (7.4)
Hexaconazole	115.0 (16.2)	85.5 (8.6)	66.5 (6.6)
Propiconazole	137.0 (14.9)	72.3 (7.1)	110.6 (56.6)
Difenoconazole	121.0 (15.8)	151.0 (6.8)	111.4 (6.1)

Table 5. Recovery of Triazoles in carrot (n=5)

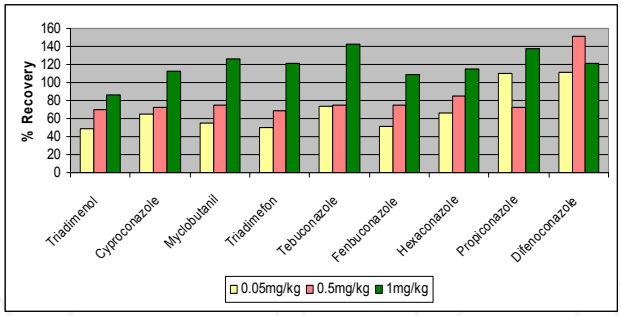

Figure 9. Recoveries fortified in Carrot (n=5 at each level)

4. Conclusion

Lower recoveries for some analytes in certain matrices and at certain concentrations in pesticide residue analysis could be due to the degradation of base sensitive pesticides in higher pH samples, or degradation of acid sensitive pesticides in lower pH samples. And protonization of basic pesticides in acidic conditions reduces partition into organic layer.

For consistent and higher recoveries, some considerations that we need to look into are the homogeneity of the samples, the choice of solvent, sorbent(s) and salt(s) used during clean-up process. The EtOAc modified QuEChERS method was demonstrated to provide

consistent and reproducible recoveries for tomato, cabbage and orange for most triazole compounds but not for carrot. High Performance Liquid Chromatography coupled with mass spectrometry by atmospheric pressure ionisation can be used for the identification of triazole fungicides in vegetables and additional confirmatory is not needed.

More sensitive analytical methods such as LC-QTof (Liquid chromatography high resolution time-of-flight), orbitrap mass analyzers (LC-HR-MS), LC-MS/MS that have higher sensitivity and specificity can also be used. LC-QTof, LC-MS/MS can be used for screening and confirmation work and need no additional confirmatory. Liquid chromatography coupled with high resolution time-of-flight or orbitrap mass analyzers (LC-HR-MS) seems to open new and attractive possibilities for residue analysis [30-31].

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5. References

[1] Wu Y. S.; Lee H. K.; and Li S. F. Y. (2001) *J Chromatography. A.*, 912, 171-179
[2] Mueller, 2006]
[3] Fillion, J., Sauvé, F., and Selwyn, J. (2000). Multiresidue method for the determination of residues of 251 pesticides in fruits and vegetables by gas chromatography/mass spectrometry and liquid chromatography with fluorescence detection. *J. AOAC Int.* 83, 698-713.
[4] S. Nemoto; K. Sasaki; S. Eto; I. Saito; H. Sakai; T. Takahashi; Y. Tonogai; T. Nagayama; S. Hor; Y. Maekawa; and M. Toyoda (2000). *J. Food Hyg. Soc. Japan* 41, 233–241 (in Japanese).
[5] H. Obana; K. Akutsu; M. Okihashi; and S. Hori (2001). *Analyst* 126, 1529–1534
[6] Anastassiades, M., Lehotay, S.J., Štajnbaher, D., and Schenck, F.J. (2003). Fast and Easy Multiresidue Method Employing Acetonitrile Extraction /Partitioning and"Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce. *J. AOAC Int.* 86 (2), 412-431.

[7] Aysal, P., Ambrus, Á., Lehotay, S.J.; and Cannavan, A. (2007). Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction. *Journal of Environmental Science and Health B*, in press (Vol.B42, No.5)

[8] Food and Drug Administration (1999) *Pesticide Analytical Manual Volume I*: Multiresidue Methods, 3rd Edition, U.S. Department of Health and Human Services, Washington, DC.

[9] Luke, M. A., Froberg, J.E., and Masumoto, H. T. (1975). Extraction and cleanup of organochlorine, organophosphate, organonitrogen, and hydrocarbon pesticides in produce for determination by gas-liquid chromatography. *J. Assoc. Off. Anal. Chem.* 58, 1020-1026.

[10] Cook, J., Beckett, M. P., Reliford, B., Hammock, W., and Engel, M. (1999) Mutiresidue analysis of pesticides in fresh fruits and vegetables using procedures developed by the Florida Department of Agriculture and Consumer Services. *J.AOAC Int.* 82, 1419-1435.

[11] Lee, S. M., Papathakis, M. L., Hsiao-Ming, C. F., and Carr, J. E. (1991). Multipesticide residue method for fruits and vegetables: California Department of Food and Agriculture. *Fresenius J. Anal. Chem.* 339, 376-383.

[12] Andersson A., and Pålsheden H. (1991). Comparison of the efficiency of different GLC multi-residue methods on crops containing pesticide residues. *Fresenius J.Anal. Chem.* 339, 365-367.

[13] Sheridan, R. S., and Meola J. R. (1999). Analysis of pesticide residues in fruits, vegetables, and milk by gas chromatography/tandem mass spectrometry. *J. AOAC Int.* 82, 982-990.

[14] Lehotay, S. J. (2000). Determination of pesticide residues in nonfatty foods by supercritical fluid extraction and gas chromatography/mass spectrometry: collaborative study. *J. AOAC Int.* 83, 680-697.

[15] Wylie, P; and Chin, K.M (2001). *Labplus International* – September/October

[16] Aguilar, C; Ferrer, I.; Borrul, F.; Marce, R.M. (1998). *J. Chromatography. A*, 794 (1/2), 147-164

[17] Aguilar, C; Penalver, S.; Pocurull, E. Borrull, F.; Marce, R.M. (1998). *J. Chromatography. A*, 795 (1), 105-116

[18] Lehotay,S.J.; Kyung, A.S.; Kwon, H.; Koeksukwiwat,U.; Fu, W.; Mastrovska, K.; Hoh, E.; and Leepipatpiboon, N. (2010). *Journal of Chromatography A.* 1217, 2548-2560.

[19] Guzetta, A (2001). Reverse Phase HPLC Basics for LC/MS, An Ion Source tutorial.

[20] Lehotay, S.J.; de Kok, A.; Hiemstra, M.; and van Bodegraven, P. (2005). Journal of AOAC International. Vol. 88, No.2,

[21] Maestroni, B.; Ghods, A.; El-Bidaoui, M.; Rathor, N.; Ton, T.; Ambrus, *A. (*2000). In *Principles and Practices of Method Validation;* Fajgelj, A., Ambrus, A.,Eds.; The Royal Society of Chemistry, Cambridge, England, 49-58.

[22] Maestroni, B.; Ghods, A.; El-Bidaoui, M.; Rathor, N.; Jarju, O.P.;Ton, T.; Ambrus, A. (2000). In *Prin-ciples and Practices of Method Validation;* Fajgelj, A., Ambrus, A.,Eds.; The Royal Society of Chemistry: Cambridge, England. 59-74

[23] Mol HGJ et al 2007

[24] Kadenczki, L.; Zoltan, A.: Gardi, I.; Ambrus, *A.:* Gyorfi, L.; Reese, G.; Ebing, W. (1992). *J. AOAC Int*., 75, *(1),* 53-61.
[25] Holstege D.M. et al., 1994
[26] Holland, P.T.; Boyd, A.J.; Malcolm, C.P. (2000) In *Principles and Practices of Method Validation;* Fajgelj, A., Ambrus, A., Eds.; The Royal Society of Chemistry: Cambridge, England,; 29-40.
[27] Lehotay, S.J. (2007). *J. AOAC mt*. *90,* 485-520.
[28] S.A. Barker, LCGC, Special Supplement, Review on Modern Solid-Phase Extraction (May 1998)
[29] S.A. Barker (2000). J. Chromatogr. A. 880, 63-68
[30] Thurman E.M; Ferrer, I,; and Fernández-Alba A.R. (2005). J Chromatogr A. 1067, *127-34*
[31] Picó, Y; and Barceló, D (2008). *Trends Anal. Chem*. 27, 821-835