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Project title	New thin-layer chromatography (TLC) based sample preparation method for pesticide analysis with liquid chromatography-mass spectrometry (LC-MS)
Student names	Mr.Wuttikorn Torprasertkul ID 6033838823
Program	Bachelor of Science in Applied Chemistry
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Faculty of Science, Chulalongkorn University

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by
Mr. Wuttikorn Torprasertkul

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Accepted by Department of Chemistry, Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science Program in Applied Chemistry (International Program)

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Department of Chemistry, Faculty of Science, Chulalongkorn University, Academic Year 2020

Abstract

In this research, a new thin layer chromatography (TLC) technique was developed as a simple and selective sample preparation approach to improve pesticide analysis with liquid chromatography hyphenated with tandem mass spectrometry (LC-MS/MS). The sample preparation was performed by sample loading and separation on a TLC plate using 3:2 v/v hexene:acetone mobile phase system followed by selective cut of the target regions and the analyte recover into acetonitrile prior to the LC-MS/MS analysis. The approach was initially investigated for removal of majority of matrices in vegetable and supplementary bee pollen samples. Further analysis was performed for the target pesticide analytes (carbaryl, chlorpyrifos and atrazine) spiked into the bee pollen sample. With 4 times loading of the sample, the method detection limits are 1.383, 0.012, and 0.001 ppm for carbaryl, chlorpyrifos, and atrazine, respectively. The method limits of quantifications are 4.611, 0.039, and 0.003 ppm, respectively. The sensitivity could also be improved by increasing sample loading amounts as illustrated for atrazine (0.01 ppm), where 10 times loading (400 μL) resulted in 9 times improved peak areas compared with the 4 times loading. The obtained results were also compared with that prepared by the conventional Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method. For the intraday repeatability, the percentages of relative standard deviations (%RSD) obtained with the TLC based approach were 10.90, 10.58, and 13.47 for carbaryl, chlorpyrifos, and atrazine, respectively, which were better than that offered by the QuEChERS method with the %RSD of 29.71, 21.67, and 18.23, respectively. The TLC approach more effectively removed the sample matrix and required lower amounts of solvent and sample. However, QuEChERS provided the higher analyte peak areas and recoveries for the investigated sample. The developed TLC method can be an alternative choice for selective sample preparation of target pesticides in food and environmental samples in the future.

Keywords: Environmental sample, food analysis, herbicide analysis, LC-QqQ-MS, TLC extraction.

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Chapter 1
Introduction

1.1 Introduction to the research problem and significance

Pesticides are chemicals that are used to protect crops and to ensure their quality, to maximize the production yield. Apart from their benefits, pesticides can be toxic to humans and other living things. Pesticides are classified into two major categories- carcinogenic (incite cancer) and non-carcinogenic. Some of the two short-term effects are headaches and nausea. Unfortunately, they may cause cancer and damage on reproductive systems. Another problem is that their use can decrease the general biodiversity of the soil, thus reducing soil quality. Bioaccumulation also occurs when an organism absorbs a significantly high concentration of toxic compounds and these enter the food chain [1].

As a result, pesticide residue analysis is required to control the level of pesticides in the environment. Currently, liquid chromatography-mass spectrometry (LC-MS) analysis is the most common method to detect pesticides and measure their detection limit. Before the LC-MS analysis, pesticide samples are usually prepared with the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method. QuEChERS is one kind of solid-phase extraction that provides high recoveries and the final extract, being dissolved in acetonitrile, brings flexibility in the choice of the determinative analysis technique. However, the QuEChERS method is costly and requires a significant amount of solvent [2].

On the other hand, thin-layer chromatography (TLC) can be a good alternative to QuEChERS due to its advantages such as fast analysis time, low solvent usage on a per-sample basis, and cost effectiveness [3]. TLC is a chromatographic technique used to separate non-volatile mixtures performed on a sheet of glass, plastic, or aluminium foil, which is coated with a layer of adsorbent material (stationary phase), normally silica gel, alumina, or cellulose.

In this work, pollen of 'western honeybee' (*Apis mellifera*) was used as a sample for pesticide residue analysis. Bee pollen is rich in flavonoids and phenolic acids, which are antioxidant compounds that help to inhibit the action of inflammatory enzymes [4]. Normally, people consume it by brewing with tea or coffee. The target analytes are widely used pesticides likes chlorpyrifos, atrazine, carbaryl, etc. which can be found in bee pollen and other agricultural products. This study aims to develop the new TLC based sample preparation approach that minimizes the use of solvent for selective trace analysis of pesticides and liquid chromatography-mass tandem spectrometry (LC-MS/MS) was employed for the pesticide analysis.

1.2 Research objectives

1. To develop a new sample preparation approach for pesticide analysis using thin-layer chromatography (TLC) method with liquid chromatography-mass spectrometry (LC-MS)

2. To compare the performance of the TLC with that of the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method

1.3 Theory and literature search

1.3.1 QuEChERS pesticides sample preparation

Pesticides are toxic compounds used to protect crops from insects. This study aims to develop QuEChERS and dispersive solid phase extraction (dSPE) clean-up technique for sample preparation and concentration enrichment of pesticide residuals. Example has been reported for analysis of 13 widely used pesticides in soil and water samples. The samples were filtered using glass and nylon filters and then analyzed using ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) using gradient elution. This method can detect and quantify down to 0.25 ng g^{-1} and 0.01 ng mL^{-1} for soil and water samples, respectively. This method can be applied for the quantification and/or qualification of residual pesticides in the environment [5].

Another research also studied 115 pesticide residues in more than 400 commercially available orange samples. The research applied LC–MS/MS with the modified acetonitrile-based QuEChERS for the sample preparation step followed by the dSPE cleanup using primary secondary amine (PSA). The limit of detection (LOD) and limit of quantification (LOQ) for the target analytes ranged from 0.001 to 0.011 mg kg^{-1} and 0.002 to 0.030 mg kg^{-1}, respectively. The research also investigated LOD of chlorpyrifos, atrazine, and carbaryl which are the target analytes in this project. Their LOD are 0.001, 0.002, 0.001 mg kg^{-1}, respectively, with the LOQ of 0.004 mg kg^{-1} for all three standards. It should be noted that LOD and LOQ vary through instrument and condition [6].

1.3.2 Separation using thin-layer chromatography (TLC)

Thin-layer chromatography (TLC) is a chromatographic technique used to separate mixtures performed on a sheet of glass, plastic, or aluminium foil, which is coated with a layer of adsorbent material (stationary phase), such as silica gel, alumina, or cellulose. TLC technique is normally used by chemists to observe reactants and products with different functional groups in organic reactions. For the confirmation of the result, the distance traveled by a spot of interest is divided by the total distance traveled by the mobile phase (at the solvent front), to calculate the factor of retardation (R_f). There will be a low R_f for a substance whose polarity is similar to the stationary

phase, whereas a substance with a polarity close to the mobile phase will have a high R_f ('Like Dissolve Like' mechanism) [7].

$$R_f = \frac{Distance\ \ traveled\ \ by\ \ solute}{Distance\ \ traveled\ \ by\ \ solvent}$$

Figure 1.1 R_f formula

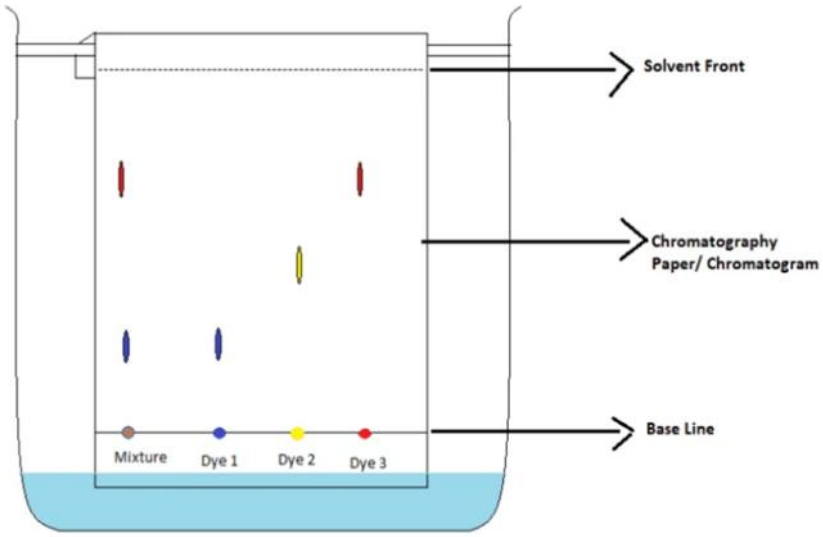

Figure 1.2 Components of TLC [8]

1.3.3 Hyphenated technique with TLC

TLC can be combined with other techniques resulting in improved capability in quantitative and qualitative analysis. TLC hyphenated with several techniques have been reported, such as comparison between the fingerprint results obtained from one-dimension low temperature TLC hyphenated with mass spectrometry (1-D LT TLC-MS) and two-dimension low temperature TLC-LC-MS (2-D LT TLC-LC-MS) for the improvement of essential oil separation derived from several species, mainly *Salvia* genus. The result showed that the 2-D LT TLC-LC-MS with reversed phase mode system gives more accurate information of complex samples than 1-DLT TLC-MS. Furthermore, the spectra obtained from 2-D LT TLC-LC-MS showed differentiation of compounds that contain isoprene units (71, 87, 89, and 126 units), whereas this could not be achieved with 1-D LT TLC-MS technique. However, both methods can be applicable for the identification and quality control of several medicinal plants [9].

1.3.4 Effects of sample matrix on LC-MS results

Although the common perception is that LC-MS offers specificity and

reliability in detection and quantitation of chemicals, LC-MS analysis does encounter some problems caused by matrix effects. A matrix is defined as components in the sample other than the target analytes (**Fig 1.3**). Matrix components include endogenous and exogenous factors. In LC-MS analysis, the matrix components with the same *m/z* ratios as that of the target analytes can be co-eluted with the analyte peak and interfere with the ionization process of the analyte in MS. This matrix effect can cause ionization suppression, which adversely affects the results of LC-MS analysis, and leads to error in analyte quantitation [10].

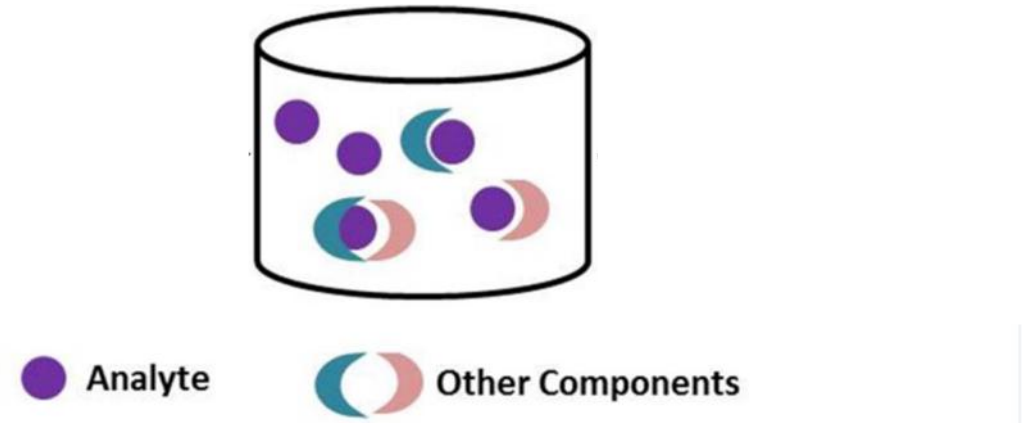

Figure 1.3 Analyte and other components (matrix) in the sample [11]

Chapter 2
Experimental

2.1 List of equipment and instrument

1. Forceps (Sigma, Germany)
2. Sigma Rectangular TLC development tank (17.5 cm×6.2 cm×6.8 cm), (Sigma, Germany)
3. 500 mL Low Form Griffin Beaker (Pyrex, France)
4. Class A 500 mL Volumetric flask (Pyrex, France)
5. 13 mm Syringe Filter Nylon membrane (0.22 micron, PRECLEAN, Thailand)
6. 2 mL Dark Brown Amber Vial (Scientific Industry, USA)
7. Centrifuge (Thomas Scientific, USA)
8. Vortex Genie 2 (Scientific Industry, USA)
9. 30 mL Centrifuge tube (Thomas Scientific, USA)
10. Laboratory Fume Hood (NDN, Thailand)
11. Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS), (Agilent, USA)
12. Micro insert (NIPRO, Thailand)
13. 1 mL Syringe (NIPRO, Thailand)
14. Scientific Oven (Scientific Industry, USA)
15. 2 µL, 10 µL, 20 µL, 100 µL, 1 mL Micropipette (Eppendorf, Germany)

2.2 List of chemicals and materials

1. Thin-layer chromatography (TLC) sheet (2×10, 4×10 cm) (Merck, Darmstadt Germany)
2. QuEChERS Kits - 'Extraction Kit' (6 g magnesium sulfate, 1.5 g sodium acetate, and 150 µL acetic acid) and 'Cleanup Kit' (1.5 g magnesium sulfate and 0.5 g PSA and 0.5 g C18 silica (Chromatific, Germany)
3. Acetonitrile reagent grade (Merck, Darmstadt Germany)
4. TLC Mobile Phase - hexene:acetone (3:2) (Merck, Darmstadt, Germany)
5. Diluent - methanol:water (MilliQ) (1:1) (Merck, Darmstadt, Germany)
6. Nitrogen gas (Linde, Thailand)
7. Sample - vegetable (*Brassica oleracea Alboglabra*) (Thailand)
8. Sample - bee pollen (from *Apis mellifera*) (Thailand)
9. LC-MS/MS Mobile Phase (A: water, 0.05% formic acid in water. B: methanol, 0.05% formic acid in methanol, Merck, Darmstadt Germany)

2.3 Optimization of TLC

2.3.1 Reference peak of standard on TLC sheet

Firstly, the 100 ppm standard stock solutions of chlorpyrifos, atrazine, and carbaryl were prepared in acetonitrile solvent. Secondly, the TLC sheet was cut into pieces with 2 cm width and 10 cm height. Thirdly, TLC tanks were prepared with a 3:2 of hexene and acetone mobile phase system. The prepared standards were spotted (40 µL) individually on the TLC sheets. After finishing the TLC separation, the sheets were dyed by dipping them into the vanillin solution (**Fig 2.1**) and heated at approximately 120 °C in an oven (**Fig 2.2**). Finally, the reference standard band on TLC was observed by naked eyes and photographed.

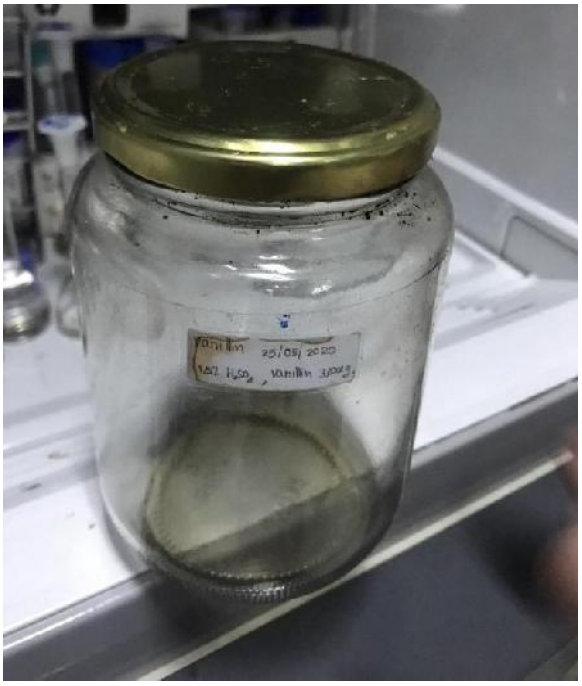

Figure 2.1 Vanillin solution

Figure 2.2 Oven used for TLC dying process

2.3.2 Sample preparation and matrix elution on TLC sheet

At Amphawa district of Samut Songkhram, pure pollen of 'western honey bee' (*Apis mellifera*) was collected using Pollen Trap (**Fig 2.3**) and kept in the cooling bag. After that, it was brought to the Department of Biology, Faculty of Science, Chulalongkorn University and kept in a refrigerator.

Figure 2.3 Pollen trap sampler

Then, 10 g of pure bee pollen and 10 g of vegetable samples were mashed separately by using a mortar (**Fig 2.4-2.5**). Next, each sample was transferred into a 30 mL centrifuge tube with the 10 mL of solvent to make a total solution of 10 g sample: 30 mL acetonitrile. Then, those were put in the 30 mL centrifuge tube, followed by 1 min vortex and centrifuging with 4500 rpm for half an hour (**Fig 2.6**). Supernatant layers of pure bee pollen and vegetable samples were spotted onto the TLC plate, separated, coated with the vanillin solution, and heated with the same method as that applied for the 3 pesticide standards.

Figure 2.4 Mashed vegetable

Figure 2.5 Mashed bee pollen.

Figure 2.6 Centrifuged vegetable and bee pollen

2.4 Analysis of standards with different concentrations for determination of LOD, LOQ and recovery with TLC and TLC/LC-MS/MS

First, 100 ppm of each pesticide standard (**Fig 2.7**) was mixed into the bee pollen sample (10%v/v atrazine, 10%v/v carbaryl, 10%v/v chlorpyrifos, and 70%v/v bee pollen, resulting in 10 ppm of each pesticide in the sample). The sample was separated by using the same TLC method as described above (3:2 hexene:acetone mobile phase), but this sample (40 µL) was loaded onto a 4x10 cm analytical TLC sheet for 4 times,

with the total volume of 160 µL. After separation of the sample for 15 min, the TLC sheet was cut at the distance of 4 cm from the loaded position, and the area from the cut position to the solvent front for further analysis.

Figure 2.7 100 ppm of chlorpyrifos, carbaryl, and atrazine

The cut paper was scraped into powder and put in a 3 mL centrifuge tube with 1 mL of acetonitrile (ACN). Then, the suspension was filtered with a membrane microfilter and a 1 mL syringe into 1.5 mL vial. After that, the filtered sample was purged with nitrogen gas for approximately 5 min to preconcentrate the analyte by evaporating ACN, with the remaining volume of roughly 200 µL. To make it applicable for LC-MS analysis, the sample was transferred into a micro insert inside a 1.5 mL vial, waiting for the LC-MS analysis. Standard concentrations were varied to result in the best linearity range with LC-MS analysis (with the concentration profiles mentioned in **Table 2.1**). Noted that Samples 1 - 7 were spotted on TLC for 4 times (40x4 = 160 µL). Sample 8 - 9 were spotted on TLC 10 times (40x10 = 400 µL). Moreover, Blank (70% bee pollen and 30% acetonitrile) and control (the standard mixtures without the bee pollen) were also analyzed.

Table 2.1 Concentration profiles of different pesticide analytes added into the blank sample resulting in different concentrations loaded on TLC.

Sample Bee Pollen	Concentration (ppm)		
	Atrazine	**Carbaryl**	**Chlorpyrifos**
Sample 1 (4 loading times)	0.010	1.000	0.002
Sample 2 (4 loading times)	0.100	2.000	0.005
Sample 3 (4 loading times)	0.200	3.000	0.010
Sample 4 (4 loading times)	0.500	4.000	0.020
Sample 5 (4 loading times)	1.000	5.000	0.030
Sample 6 (4 loading times)	0.001	2.000	0.040
Sample 7 (4 loading times)	0.005	3.000	0.050
Sample 8 (10 loading times)	0.01	1.000	0.002
Sample 9 (10 loading times)	0.001	0.500	0.005
Blank (70%v/v Bee Pollen + 30%v/v ACN)	-	-	-

2.4.1 Precision

After obtaining the best linearity range, the TLC based sample preparation and LC-MS steps were repeated for the samples containing chlorpyrifos, atrazine, and carbaryl with the concentrations of 0.02, 0.001 and 2 ppm. Those were repeated interday and intraday (3 times per day and 3 days in a row) to check the accuracy and precision of the developed method.

2.4.2 QuEChERS method

To begin with, 10.50 mL of supernatant layer of the prepared bee pollen sample was transferred to the centrifuge tube. Then, 1.500 mL of chlorpyrifos, atrazine, or carbaryl was added into the tube resulting in concentrations of 0.020, 0.001 or 2.000 ppm, respectively. After that, the mixture underwent 3 min vortex and addition of ACN until a total volume of 30 mL was obtained and transferred to 'Extract Kit Tube' (**Fig 2.8**). After that, added 150 µL of acetic acid and centrifuge with 3000 rpm for 15 minutes.

After that, 10 mL of the supernatant layer was transferred into 'Cleanup Kit Tube' (**Fig 2.9**), followed by 3 min vortex and the centrifuge. Later on, 6 mL of supernatant layer was cleaned by Syringe Filter Nylon membrane, and transferred to 15 mL tube (weighted '15 mL tube' and '15 mL tube with sample'). Then, the solution was purged with nitrogen gas until reaching 0.5 mL and transferred into a 1.5 mL vial for further LC-MS analysis.

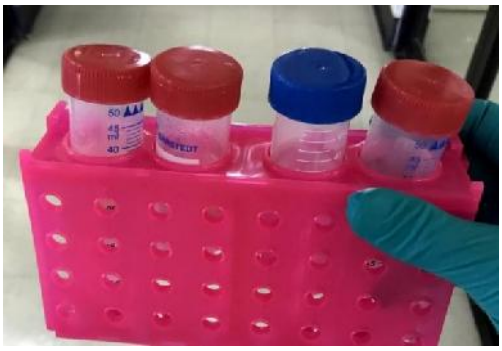

Figure 2.8 QuEChERS 'Extract Kit Tube'

Figure 2.9 QuEChERS 'Cleanup Kit Tube'

2.4.3 Liquid chromatography- tandem mass spectrometry (LC-MS/MS)

Firstly, the LC-MS/MS mobile phase was prepared into four "1000 mL DURAN bottles": 1. mobile phase A: 500 mL water, 2. mobile phase A: 0.05%v/v formic acid in 500 mL water, 3. mobile phase B: 500 mL methanol, and 4. mobile phase B: 0.05%v/v formic acid in 500 mL methanol. A sample called diluent was also prepared with a 1:1v/v ratio of methanol to water.

Samples were analyzed by using an Agilent 1220 Infinity LC system (Agilent Technologies, **Fig. 2.10**) with a reversed-phase ZORBAX Eclipse XDB-C18 column (2.1 mm × 150 mm, 3.5 μm particle diameter; Agilent Technologies, USA) and a flow rate of 0.3 mL/min. Separation was performed at room temperature (approximately 25 °C). Gradient elution was performed (as shown in **Table 2**). The column was equilibrated at 10 % v/v B for 5 min with the overall analysis time of 25 min before the next analysis. The eluted compounds were detected by mass spectrometry (MS) using electrospray ionization (ESI) which produces ions by spraying at high temperature with the drying gas.

Table 2.2 LC-MS/MS gradient elution condition**.**

Time (min)	Mobile Phase A	Mobile Phase B
0-2	80%	20%
2-12	40-80%	20-60%
12-30	0-40%	60-100%
30-35	0%	100%

Figure 2.10 Liquid chromatography- tandem mass spectrometry (LC-MS/MS)

Chapter 3
Results and discussion

The results in **Fig. 3.1** shows the bands of reference pesticides standards elution on a 2x10 cm thin-layer chromatography (TLC) plate. Atrazine showed two separated bands obviously (on at 6.0 cm ($R_f = 0.75$) and 6.3 cm ($R_f = 0.78$) away from the loaded baseline position, while carbaryl revealed only a single band at 5.6 cm ($R_f = 0.7$) from baseline. On the other hand, Chlorpyrifos' elution was not clearly detected at around 6.1 cm ($R_f = 0.76$) from the origin. All the studied pesticides eluted at the top half of the TLC plate. This observation corresponds to the significantly high hydrophobicity of these pesticides as indicated by the log K_{OW} values of about 3, 2 and 4 for atrazine, carbaryl and chlorpyrifos, respectively. This also suggests that they can be selectively analyzed by cutting the corresponding regions gave a clear idea where pesticides will elute, so this suggests the right position on the TLC sheet, containing target analyte prior to for the next analysis step.

Figure 3.1 TLC analysis results of three pesticide standards (100 ppm each) with 40 µL of the loaded volume: chlorpyrifos, carbaryl, atrazine from left to right, respectively

3.1 Matrix elution on TLC

For the vegetable samples, the eluted spot band was very broad after the separation (**Fig. 3.2**). However, most of the components eluted at the bottom half of the TLC plate as we can observe the green-yellow color, even without UV lamps and

vanillin dying solution. This is due to the sample mostly containing the major components of polymeric needed because the sample contains chlorophyll pigments which could only elute within a short distance from the loaded position. This can suggests that the matrix interference can be clearly removed from the sample by cutting off the bottom half of the TLC plate which is not our target analyte in the sample.

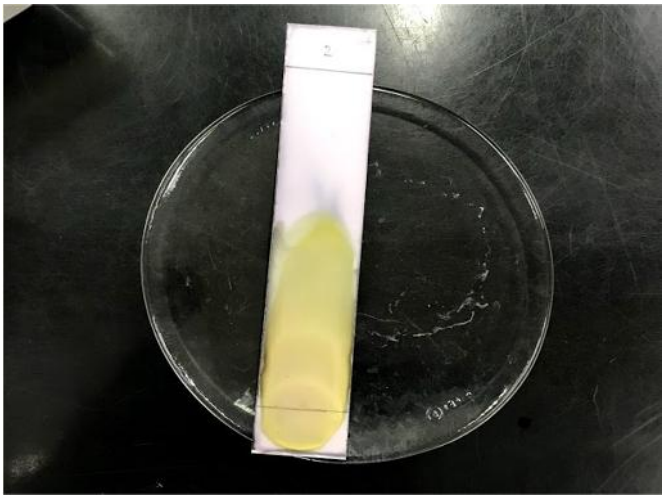

Figure 3.2 TLC analysis result of the vegetable sample with 40.00 µL of the loaded volume

Figure 3.3 TLC analysis result of the bee pollen sample with 40.00 µL of the loaded volume

From the bee pollen sample, there was a matrix elution both at the top and bottom parts of the TLC after separation (**Fig. 3.3**). There may be our target pesticides inside this sample or other hydrophobic matrix since the bands were observed at the top half where the target pesticides are located. However, the band color was mild

suggesting their low concentration. Another possibility is that the bee pollen sample may contain other hydrophobic compounds or matrix that eluted closely to the analytes.

3.2 TLC-LC-MS/MS analysis

Referring to the research paper using different separation conditions, the retention order was of carbaryl < atrazine < chlorpyrifos [12]. This is in agreement with that observed in this project with the observed retention times of 9.2, 9.6 and 11.4 min, respectively. **Table 3.1** shows peak area of each pesticide which was loaded with different concentrations onto the TLC plate, separated and selectively cut, recovered and then analyzed with LC-MS/MS.

Table 3.1 Peak area profile of the pesticide analytes with different concentrations (4 spot) added into the bee pollen sample.

Chlorpyrifos		**Atrazine**		**Carbaryl**	
Concentration loaded on TLC (ppm)	**Peak area (Count.s)**	**Concentration loaded on TLC (ppm)**	**Peak area (Count.s)**	**Concentration loaded on TLC (ppm)**	**Peak area (Count.s)**
0.010	26	0.001	569	1.000	13
0.020	35	0.010	820	2.000	257
0.030	66	0.100	13001	3.000	435
0.040	37	0.200	33095	4.000	256
0.050	96	0.500	40562	5.000	403
		1.000	126398		

3.2.1 Effect of increased sample loading

Table 3.2 Peak area profile of the pesticide analytes with different concentrations (10 spot) added into the bee pollen sample.

Chlorpyrifos		**Atrazine**		**Carbaryl**	
Concentration loaded on TLC (ppm)	Peak area (Count·s)	Concentration loaded on TLC (ppm)	Peak area (Count·s)	Concentration loaded on TLC (ppm)	Peak area (Count·s)
0.002	0	0.010	7502	1.000	0

For the chlorpyrifos and carbaryl, those are in accordance with 4 spots samples, none peak area was found. For atrazine, with an increase in 10 times concentration (0.01 ppm loaded), abundant peak area was observed (7502 count.s), while 4 spots only obtained a small area (820 count.s). This informs that 10 spots with

a total spot volume of 400 microliter**,** is suitable for the analysis of atrazine involved samples.

3.2.2 Method validation

3.2.2.1 Linearity of the calibration curves

The calibration curves of the investigated pesticides are shown in **Fig.3.4**.

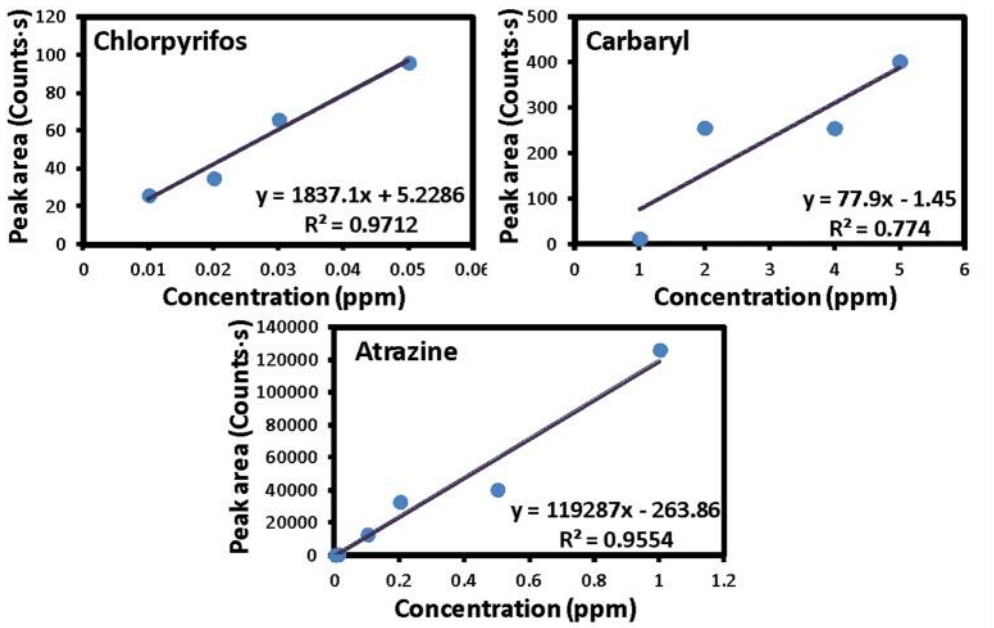

Figure 3.4 Calibration curves of chlorpyrifos, carbaryl, and atrazine obtained by using TLC/LC-MS/MS approach

3.2.2.2 Precision

Referring to the R^2 of chlorpyrifos and atrazine which were 0.971 and 0.955, respectively, those are acceptable in terms of R^2 in the linear line and good linearity relationship, making it applicable for both quantitative and qualitative analysis.

In contrast, carbaryl gave only 0.774 for R^2, relating that this data was an unacceptable linearity relationship. The error may have occurred due to the unstable TLC method somewhat it is difficult to control. For instance, it is a difficult tasks to scrape the surface (silica layer) and it is very subjective to control the amount of acetonitrile that can evaporate out during the experiment prior to LC-MS/MS analysis.

For the intraday repeatability, standard deviations of the results are 7.09 count.s, 35.92 count.s, and 41.67 count.s for chlorpyrifos, carbaryl, and atrazine, respectively with the relative standard deviations (%RSD) of 10.58%, 10.90%, 13.47%, respectively. Within the day, these SD and low %RSD are acceptable in terms of statistical point of view. When repeated, the values do not fluctuate much from the average value.

On the other hand, for the interday repeatability, the %RSD values were 32.73%, 25.93%, and 36.98% for chlorpyrifos, carbaryl, and atrazine, respectively. The results were poor. These phenomena implied that the method will strongly fluctuate between the day.

3.2.2.3 LOD and LOQ

The limits of detection were calculated for the pesticides using (3×intraday S.D.)/(slope of the calibration curve). The values were 1.383, 0.012, and 0.001 ppm for carbaryl, chlorpyrifos, atrazine, respectively. The corresponding LOQ were also approximated using (10×intraday S.D.)/(slope of the calibration curve). The values were 4.611, 0.039, and 0.003 ppm, respectively.

3.3 Comparison with the other sample preparation techniques

3.3.1 Direct dilution and QuEChERS method

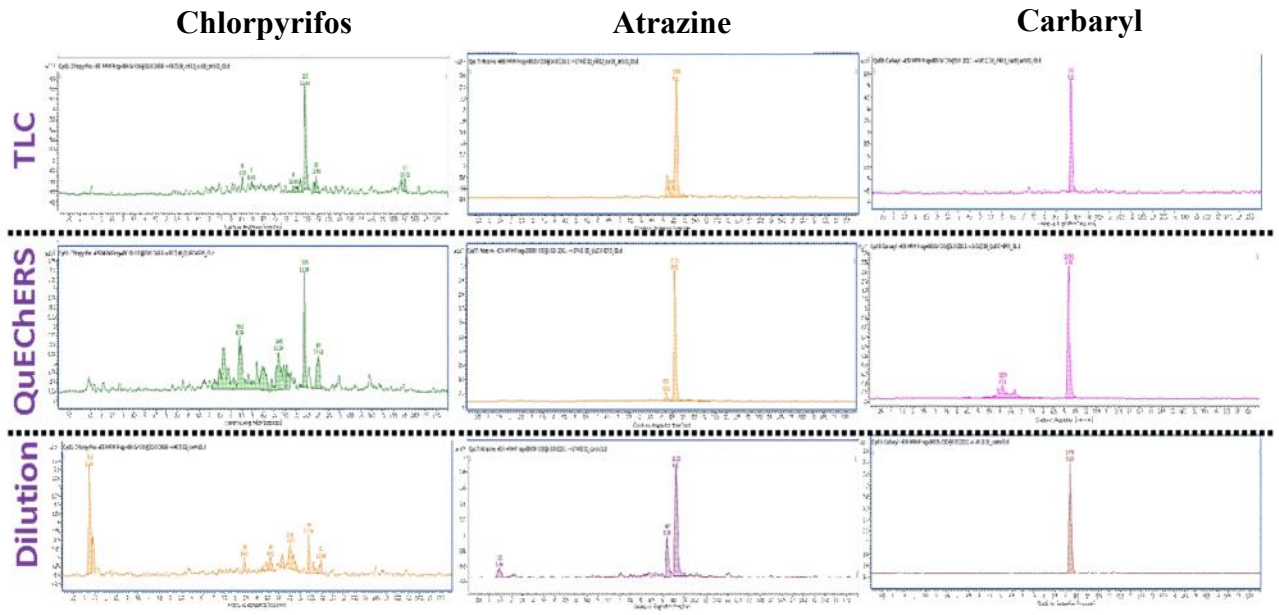

Figure 3.5 LC-MS/MS results of the bee pollen sample added with chlorpyrifos, atrazine, and carbaryl with the concentrations of 0.020, 0.001, and 2.000 ppm, respectively, prepared by TLC, QuEChERS, and direct dilution approach.

The corresponding MS/MS transitions were 349.9➔96.7 *m/z*, 216.1➔174.0 *m/z* and 202.1➔145.1 *m/z*, respectively. Direct dilution approach clearly resulted in higher matrix interference as well as the other matrices and components not detected under the selective MS/MS transition which could shorten the instrument lifetime.

For the sample preparation using QuEChERS, the obtained peak gives a high recovery because of the high peak area. However, this method requires more solvent

than the TLC method. It should be noted that matrix interference can still be observed in chlorpyrifos and carbaryl chromatograms shown in Fig 3.5.

On the contrary, TLC sample preparation can remove the majority of the matrix. As a result, it provides an overall cleaner peak as shown in the figure, compared to direct dilution approach and QuEChERS method with less complicated steps. TLC gains another advantage of less solvent and cost effectiveness. Moreover, only trace the amount of sample is required for each sample analysis. Thus, the researcher can minimize the risk of contact with dangerous substances.

The QuEChERS method was performed in triplicate in order to investigate the precision of the method. Standard deviations of chlorpyrifos, carbaryl, and atrazine are 32.39 count.s, 2,480.82 count.s, and 1,134.00 count.s, respectively. Also, the %RSD also obtains using (SD/Mean)*100, which are 21.67%, 29.71%, 18.23% for chlorpyrifos, carbaryl, and atrazine, respectively. Compared to the triplication of TLC (intraday) (see section 3.2.2.2), the results were poorer. However, it is still acceptable because the variation is still not fluctuating much from the mean value.

Chapter 4
Conclusion

In conclusion, a new TLC based sample preparation technique was established for improved target pesticide analysis in food and environmental samples. The application was demonstrated for selective analysis of chlorpyrifos, atrazine, and carbaryl, commonly found in bee pollen and other agricultural products, with LC-MS/MS. The obtained results were compared with that from QuEChERS sample preparation. Although QuEChERS resulted in the higher peak areas of the pesticides in the same sample, it required larger amount of solvent and sample than the developed TLC method. The lower sensitivity of the TLC method could be improved by increasing the amount of sample loading. From LC-MS/MS results, the TLC method also illustrated greater capability to remove the sample matrix than that of QuEChERS. This informs that TLC sample preparation can be more selective and effective method for sample cleanup prior to LC-MS/MS or other chromatographic techniques. Furthermore, the intraday repeatability with the TLC approach is better with the lower %RSD than QuEChERS. The developed approach can thus be considerably simpler and "Greener" with lower amount of solvent and sample consumption which is applicable for pesticide residual analysis and other related compounds in the future.

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Biography

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