

**ANALYSIS OF DIFENOCONAZOLE, DIMETHOATE,
CHLORANTRANILIPROLE, PYMETROZINE
RESIDUES ON BRINJAL FRUITS COLLECTED FROM
DIFFERENT FIELDS AND MARKETS OF
BANGLADESH**

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**DEPARTMENT OF AGRICULTURAL CHEMISTRY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207**

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A Thesis

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CERTIFICATE

*This is to certify that thesis entitled, “Analysis of Difenconazole, Dimethoate, Chlorantraniliprole, Pymetrozine residues on brinjal fruits collected from different fields and markets of Bangladesh” submitted to the, Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.) IN AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bona fide research work carried out by **MD. JUBAYER IBN YOUSUF**, Registration No.: 20-11122 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly been acknowledged.

Dated: June, 2022
Place: Dhaka, Bangladesh

Dr. Md. Tazul Islam Chowdhury
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*DEDICATED
TO
MY BELOVED PARENTS*

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Abstract

The study was conducted to analyze difenoconazole, dimethoate, chlorantraniliprole, pymetrozine residues on brinjal fruits collected from different fields and markets of Bangladesh during November 2021. The collected samples were analyzed using modified Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and LC-MS/MS for the determination of pesticide residues in 50 field samples and 50 market samples of brinjal. Among the 50 analyzed field samples, pymetrozine was detected in 4 samples (8%), dimethaote was detected in 10 samples (20%), difenoconazole was detected in 6 samples (12%) and chlorantraniliprole was detected in 7 samples (14%). In 50 market samples, pymetrozine was detected in 2 samples (4%), dimethaote was detected in 5 samples (10%), difenoconazole was detected in 4 samples (8%) and chlorantraniliprole was detected in 8 samples (16%). All of the samples detected with pymetrozine, dimethaote, difenoconazole residues has residue above Maximum Residue Level (MRL).

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
ADI	Acceptable Daily Intake
A.I.	Active Ingredient
ARfD	Acute Reference Dose
BCSIR	Bangladesh Council of Scientific and Industrial Research
BDL	Below Detection Limit
CLP	Clorantraniliprole
DFN	Difenoconazole
DMT	Dimethoate
ECD	Electron Capture Detector
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
ESI+	Positive Electrospray Ionisation
FTD	Flame Thermionic Detector
GAP	Good Agricultural Practice
GC	Gas Chromatography
GCB	Graphitised Carbon Black
GLC	Gas-Liquid Chromatography

HPLC	High Pressure Liquid Chromatography
HPLC-DAD	High-Performance Liquid Chromatography with Diode-Array Detection
INARS	Institute of National Analytical Research and Services
LC	Liquid Chromatography
LC-MS/MS	Liquid Chromatography with tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MRL	Maximum Residue Level
MRM	Multiple Reaction Monitoring
MSPD	Matrix Solid Phase Dispersion
OC	Organochlorine
OP	Organophosphate
PHI	Pre-harvest interval
PSA	Primary Secondary Amine
PYM	Pymetrozine
QQQ	Triple Quadruple LC-MS/MS
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RQ	Risk Quotient
RP-HPLC	Reverse Phase High Performance Liquid Chromatography

RSD	Relative Standard Deviation
SC	Suspension concentrate
S/N	Signal-to-noise ratio
UHPLC	Ultra-High-Performance Liquid Chromatogra

CHAPTER 1

INTRODUCTION

Brinjal (*Solanum melongena*) is an important, inexpensive, and popular vegetable in Bangladesh. It is highest produced vegetable after potato in Bangladesh. According to the Bangladesh Bureau of Statistics, brinjal is the second most important vegetable grown in both the summer (9.6% of total vegetable production) and winter (4.7% of total vegetable production) seasons of Bangladesh. Brinjal is common in our daily diet (7.28 g/person-day) and is served all around the year (Bushra et al., 2022). According to Naeem and Ugur, dietary intake of brinjal fruits supplies a significant amount of an adult's daily need for vitamins, minerals, and phenolic compounds (Naeem and Ugur, 2019).

Vegetables play a significant part in Bangladeshi agriculture by supplying food, nutrition, and economic security. But it is contributing more significantly by yielding higher returns per unit area and time. Our nation's agriculture is transitioning from the production of rice for sustenance to higher-value crops like vegetables and fruits. Vegetable crop diversification and rising commercialization can support the growth of the agriculture industry in a number of ways. The dependence of the majority of vegetable producers on the heavy application of pesticide is one possible disadvantage connected to a shift toward more intensive vegetable production (Hossain, 2000). Vegetable production in Bangladesh is growing day by day. After China and India, Bangladesh is the third-largest vegetable producer in the globe. Cabbage, cauliflower, tomato, brinjal, potato, radish, carrot, amaranth, green banana, green papaya, bulbous root of arum, taro stem, country bean, bottle gourd, pumpkin, bitter gourd, teasle

gourd, ribbed gourd, ash gourd, okra, yard long bean, spinach, etc. are vegetables that are frequently produced in Bangladesh (Hasan et al., 2017).

Pesticide application is one of the most important innovations in crop production (Ghimire and Woodward, 2013). In modern agriculture, the use of pesticides to protect the crops from different pests and diseases has become evident (Juraske et al., 2007). Use of pesticide directly contributes in farm productivity. Being most affordable, brinjal is known as “common man's vegetable” grown in almost all over Bangladesh. Brinjal contains very low amount of saturated fat, cholesterol and sodium. It is also a good source of Vitamin C, Vitamin K, Thiamin, Niacin, Vitamin B6, Pantothenic Acid, Magnesium, Phosphorus, Copper, Dietary Fiber, Folate, Potassium and Manganese. In Bangladesh, production of brinjal was 558 thousand metric tons during 2019–2020 (*Statistical Yearbook Bangladesh 2020*, n.d.). But, fresh vegetables have higher potentiality to contain residue of harmful and toxic pesticides. Which is a health risk factor for consumers. Thus, food safety has become a major public concern worldwide (Radwan and Salama, 2006). Therefore, application of pesticides at optimum level should be ensured due to their relative toxicity to the environment and human health (Jiang et al., 2009).

Nowadays, consumers misperceptions about the differences between the perceived toxicity and the actual risk of pesticide residues has become a major concern (Cao et al., 2017). Many researchers are investigating the pesticide residue levels in vegetables to evade possible risks of toxicity to human health (Osman et al., 2010). Regulatory agencies worldwide have established Maximum Residue Limits (MRL) for food commodities to ensure food safety and facilitate international trade. MRLs are a trading standard and a measure of the highest legally permitted level of a pesticide

residue on food, agricultural commodities, or animal feed that are based on sound agricultural practices (e.g., Integrated Pest Management, a sustainable control strategy including rational application of pesticides to minimize residues). MRLs are set well below safety margins to ensure food safety for producers (farmers), traders, regulators, and ultimately, consumers (MacLachlan and Hamilton, 2010). Pesticide residue analysis is the critical first step in applying these standards (Picó et al., 2006).

Difenoconazole is also known as *cis-trans-3-chloro-4-(4-methyl-2-(1H-1,2,4-triazol-yl methyl)*, a typical fungicide with a substituted triazole moiety that binds the heme part of the fungus cytochrome P450(cyp)51-is-1,3-dioxolan-2-yl)phenyl4-chlorophenyl ether (Vanden Bossche et al., 1990). It suppresses the action of the fungus'lanosterol-14-ademethylase enzyme and prevents the manufacture of ergosterol, which prevents the production of chitin for the fungus' cell walls and causes cytoplasm to overflow (Buchenaer, 1995). It is frequently used to control diseases brought on by various types of pathogenic fungus on different crops and has both protective and curative properties (Munkvold et al., 2001).

Difenoconazole research is currently mostly concerned with how different strains respond to it (Rekanovic et al., 2010). Difenoconazole has an unavoidable harmful influence on the environment despite having potent and broad-spectrum bactericidal characteristics due to its widespread use in fruits, vegetables and cereals (Gopinath et al., 2006).

Dimethoate ([O,O-Dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate]) is an organophosphorous insecticide that is used worldwide in agriculture and urban areas due to its high efficacy and rapid environmental degradation. Since its

registration in 1962, it has been used to control a variety of insects, including plant hoppers, mites, and flies (Mirajkar and Pope, 2005).

Chlorantraniliprole [3-bromo-N-[4-chloro-2-methyl-6-[(methyl amino) carbonyl] phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide] is a insecticide belonging to the anthranilic diamide class of chemistry. For synthetic insecticides known as ryanodine receptor activators, this substance possesses a brand-new method of action. In order for muscles to work properly, these receptors are essential. Chlorantraniliprole displays outstanding selectivity and safety for mammals due to structural differences between insect and mammalian ryanodine receptors (Malhat, 2012).

Pymetrozine, also known as 4,5-dihydro-6-methyl-4(3-pyridylmethyleneamino)-1,2,4-triazin-3(2H)-one (IUPAC), is a new insecticide having specific activity against homopteran insects. It is the first and sole compound in the azomethine pyridine group. Pymetrozine has been shown in numerous studies to be beneficial in integrated pest management programs against aphids, whiteflies, and plant hoppers. It exerts its distinctive effects by interfering with the neural system's control of eating behavior, which causes the insect to starve to death within a few days. It is thus one of the insecticides that could replace organophosphates. However, the United States Environmental Protection Agency (EPA) has designated pymetrozine as a "likely" human carcinogen since experimental tests found that male and female rats and mice developed benign liver hepatoma and carcinoma, two different forms of tumors. MRLs for pymetrozine in vegetables have been set in many nations, including the USA, EU member states, and Japan. These MRLs range from 0.02 to 2 mg kg⁻¹, and manufacturers recommend pre-harvest intervals of 7 to 21 days for vegetables and

other crops. As previously stated, among other things, estimations of MRLs and pre-harvest periods rely on the analysis techniques utilized and the meteorological circumstances in which the pesticides are administered (Shen et al., 2009).

LC-MS/MS is an analytical technique that combines liquid chromatography with mass spectrometry to provide highly sensitive and selective analysis. A sample solution containing analytes is pumped through a stationary phase using a mobile phase. Chemical interactions between the sample components, stationary phase, and mobile phase cause different migration rates through the LC column, resulting in separation. The combination of various stationary phase and mobile phase options allows for customization of the separation for complex solutions. After elution from the LC column, the effluent is directed to the mass spectrometer, where the LC column effluent is nebulized, desolvated, and ionized to create charged particles. These charged particles migrate through a series of mass analyzers using electromagnetic fields. The first quadrupole targets a specific mass/charge precursor ion, excluding all other mass/charge ratio particles. In the collision cell, the selected mass/charge ions are then fragmented into product ions by collision with an inert gas. The third quadrupole targets specific product ion fragments, and the resulting isolated product ions are quantified with an electron multiplier. The transition of ions from the precursor to product ion is highly specific to the compound's structure and provides a high degree of selectivity. The strength of this technique lies in the separation power of LC combined with the MS's ability to quantify compounds with high sensitivity and selectivity based on their unique mass/charge (m/z) transitions (García-Vara et al., 2022).

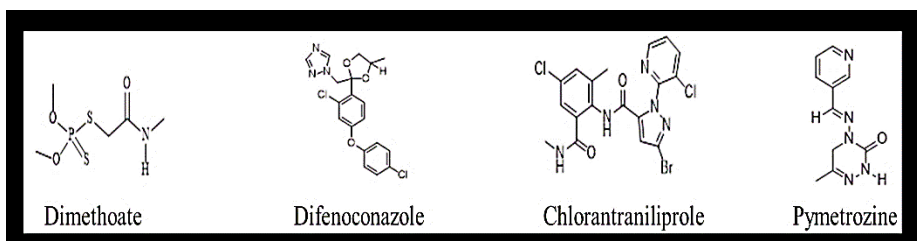


Figure. 1. Structures of four pesticides

OBJECTIVES

- To identify different pesticides present in brinjal collected from fields and markets of Bangladesh.
- To measure the amount of detected pesticide residues (mg/Kg) remain in brinjal samples.
- To compare the amount of detected pesticide residues (mg/Kg) found in brinjal samples with the Maximum Residue Limit (MRL).

CHAPTER 2

REVIEW OF LITERATURE

In this chapter challenge has been made to review literatures for updating the information regarding the current status of research and knowledge about determination of pesticide residues in brinjal. Available and accessible sources of information have been thoroughly reviewed and summarized with critical comments as appropriately as possible. Although there have been limited source of information, most of the relevant information available in and around Bangladesh was collected and reviewed. It is found that most of the information on the aspects searched as mentioned above are mostly available from research station and information of farmers' field condition are scanty. However, a significant number of study-reports on pesticides residues in brinjal are available. The studies on the quantification of detected pesticides residues below or above the Maximum Residue Limit (MRL) of vegetables in Bangladesh are rarely reported. With this background, the information collected from different sources have been reviewed and presented below:

Akhtar et al. (2018) conducted a study to determine selected pesticides namely bifenthrin, difenoconazole, paraquat, dimethomorph, imidacloprid, deltamethrin residual in fruit (guava) and vegetables (egg plant and round guord) collected from shops in commercial market, Lahore. The samples were prepared and submitted to high pressure liquid chromatography (HPLC) for the purpose of detecting pesticide residues. According to the findings, there were 5.13, 81.5, 6.6, 0.48, and 1.65 mg/kg, respectively, of bifenthrin, difenoconazole, paraquat, diomethomorph, and imidacloprid in the guava fruit. While imidacloprid residues were not found,

bifenthrin, difenoconazole, paraquat, diomethomorph, and deltamethrin were all detected in the egg plant sample at levels of 3.53, 5.62, 4.58, 0.25, and 0.005 mg/kg, respectively. Bifenthrin, difenoconazole, paraquat, and diomethomorph residues in round gourd were 3.87, 61.53, 5.01, and 0.15 mg/kg, respectively. However, fruits and vegetables still contain pesticide residues that may be harmful to consumers' health. Therefore, it is very advised to monitor pesticide residues continuously.

Xu et al. (2017) developed a sensitive and effective method, using gas chromatography (GC) and an electron capture detector (ECD), for the simultaneous quantitative determination of bifenthrin, chlorothalonil, cyfluthrin, cypermethrin, difenoconazole, fenvalerate, procymidone and pyridaben residues in cowpea. Using acetonitrile for extraction, a graphitized carbon black/amino solid phase extraction cartridge for purification, and GC-ECD for determination. A total of three spiking levels (0.01, 0.1, and 0.5 mg/kg) were used for the recovery experiments. The average recoveries for the three spiked levels ranged from 76.6 to 107.0 %, with relative standard deviations for all analytes between 1.2 and 5.6%. Each pesticide had a quantification limit of 0.01 mg/kg, which was less than or equivalent to the applicable MRLs established by China, the Codex Alimentarius, or the European Union. In comparison to existing approaches, the new analytical method was more practical, cost and time efficient, environmentally friendly, and easily accessible.

Prodhan et al. (2015), where they use a rapid, precise and efficient method for the determination of seven insecticides (chlorpyrifos, dimethoate, deltamethrin, thiamethoxam, thiacloprid, pirimicarb and indoxacarb) and three fungicides

(azoxystrobin, fluopicolide and propamocarb hydrochloride) in melon by employing quick, easy, cheap, effective, rugged and safe extraction method coupled with liquid chromatography triple quadrupole mass spectrometry which was developed and validated by evaluating the accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ). When they examined the matrix impact, they discovered that it was significant for the drugs thiamethoxam and deltamethrin (-53% and +135%, respectively). Finally, 122 fresh melon samples taken from several markets in Thessaloniki, Greece, were residue-analyzed using this approach. 32 samples (or 26% of the total number of samples) out of the 122 that were examined contained pesticide residues. None of the samples had residual levels that were higher than those set by the European Union. Fluopicolide and thiamethoxa were the insecticides that were most commonly found.

Gong et al. (2019) developed a simple determination method for pymetrozine and its metabolites in Chinese kale using liquid chromatography with tandem mass spectrometry. The approach was accurate (recoveries of 73.2–94.1%), precise (relative standard deviation of 2.5–9.8%), and linear ($R^2 > 0.99$). According to field tests, pymetrozine's half-lives in Chinese kale were 3.0-4.1 days, and during harvest, all terminal residue quantities were below the maximum residue level set by the US EPA (250 g/kg). Pymetrozine is unlikely to cause serious health problems in people when used according to advised application instructions because its risk quotient is 100%. Additionally, the impact of home processing on the presence of pymetrozine residues in Chinese kale was observed. Four methods had processing factor values ranging from 0.19 to 0.60, indicating that they could eliminate pymetrozine residues from Chinese kale, particularly pickling after washing.

Halawa (2020) conducted a research to estimate the residues of pymetrozine and penconazole in pea fruits using GC-ECD and HPLC-DAD with QuEChERS method. A knapsack sprayer with one nozzle was used to apply the substance. After an hour, 1, 3, 5, 8, 11, 15, and 21 days of treatment, samples were collected. Data obtained showed that the residual levels of penconazole and pymetrozine were both within the maximum residue limits (MRLs) set by the Codex Alimentation Commission (0.02 mg/kg and 0.05 mg/kg, respectively). According to the data, penconazole residues on pea fruits exhibited higher persistence ($t = 3.6$ days) than pymetrozine residues ($t_{12} = 2.5$ days), respectively. Pymetrozine and penconazole had pre-harvest intervals (PHI) of 8 and 12 days following treatments for pea fruits, respectively. The results of home processing of pea fruits (washing in water, peeling, and boiling) showed that there are several safe ways to reduce the levels of pymetrozine and penconazole residues.

Vijayasree et al. (2013) conducted a field trial to study the dissipation kinetics of chlorantraniliprole 18.5 % SC on cowpea fruits. By using tandem mass detection (LC-MS/MS) and liquid chromatography, the fruit samples that were periodically collected were examined. According to first order kinetics, the first residues of chlorantraniliprole in cowpea fruits were found to be 0.55 mg/kg, but they quickly disappeared with a half-life of 1.31 days and a waiting period of 0.62 days. The pesticide residues on cowpea fruits gathered two hours after spraying were reduced by 47.19%-91.70% and by 44.56%-91.25% on fruits picked on the third day, respectively, when the fruits were processed using domestic treatments.

Kar et al. (2013) carried out a research to study the dissipation pattern of chlorantraniliprole on cauliflower and to suggest suitable waiting period for the safety of consumers. Quick, easy, cheap, effective, rugged, and safe method was used for the extraction and cleanup of samples and the residues of chlorantraniliprole were estimated using high-performance liquid chromatograph (HPLC) and confirmed by liquid chromatograph–mass spectrometer and high-performance thin layer chromatograph. The average first deposits of chlorantraniliprole (Coragen 18.5 SC) were found to be 0.18 mg/kg and 0.29 mg/kg, respectively, after three applications at the prescribed dose (9.25 g a.i. ha⁻¹) and double the indicated amount (18.50 g a.i. ha⁻¹). The maximum residue level of 2.0 mg/kg imposed by the Codex Alimentarius Commission was determined to be lower than these deposits. At recommended and double the recommended dosages, these residues disappeared after 3 and 5 days, or below the quantitative limit of 0.10 mg/kg. When used at acceptable quantities, chlorantraniliprole's half-life value ($T_{1/2}$) was calculated to be 1.36 days. As a result, it appears that using this insecticide in the recommended amounts does not provide any harm, and a one-day waiting period is advised before consuming cauliflower curds.

Nakajima et al. (2018) measured the residual amounts of chlorantraniliprole in various vegetables and fruits. The QuEChERS method was used to create sample solutions, which were then analyzed by LC-MS/MS in accordance with our standard procedure. By using recovery tests with five repetitions and a concentration of 10 ng/g, performance characteristics for eight different types of meal samples were assessed. The percentage ranges for recoveries and RSDs were respectively 50.2 to 93.4% and 2.1 to 9.7%. Utilizing this strategy to survey 163 fruits and 207 vegetables resulted in detection rates of 8.2 and 1.2%, respectively. Okra (4 out of 10 tests), paprika (4 out

of 23 samples), and tomato (2 out of 6 samples had high detection rates in vegetables, and leafy vegetables such lettuce, mizuna, spinach, and wrinkled greens also had high levels of chlorantraniliprole. In mizuna, the maximum residual concentration was 571 ng/g. The samples that included chlorantraniliprole appeared to be primarily from Asian nations, including samples that were originally from Japan. But none of them had levels over the MRL, indicating that chlorantraniliprole use has been carefully monitored.

Pal et al. (2016) performed a research on “Determination of the organophosphorus pesticide in okra crop, *Abelmoschus esculentus* (L.) by gas-liquid chromatography in Meerut region.” A multi pesticide residues analysis was done for farmgate okra fruits samples from four markets places of Meerut region. Dimethoate, monocrotophos, and chlorpyrifos were examined in the samples. GLC used a multi-residue analysis to check the pesticide residues in vegetable samples of okra. According to the results, 39.0% of the samples contained pesticide contamination with residue levels below MRLs. The findings showed that 42% of the samples examined had residue levels above MRLs and 19% below BDL. A more thorough monitoring investigation involving okra from 30 various farmgate and market Meerut locations of the city is suggested in order to determine the precise location of pesticide residues based on the observations obtained in these studies.

Anwar et al. (2011) conducted a research on “Determination of pesticide residues in fruits of Nawabshah district, Sindh, Pakistan.” Eight fruit samples (apple, guava, orange, grapes, pear, persimmon, banana, and pear) were gathered for this study from

the neighborhood markets in the Nawabshah district of Sindh. Gas chromatography was used to monitor the pesticide residues of organophosphate (OP), pyrethroid, and organochlorine (OC), including dichlorvos, fenvalerate, dimethoate, methyl parathion, fenitrothion, cypermethrin, endosulfan, deltamethrin, mevinphos, chlorpyrifos, profenofos, and dicofol, in (GC). With the exception of bananas, all fruit samples were contaminated with pesticide residues, and only apple samples were found to exceed the MRL set by the Codex Alimentarius Commission.

Bagyalakshmi et al. (2011) conducted a research to determine the residue of Dimethoate in leafy vegetables (Spinach) using RPHPLC. For processed (washed, washed, and cooked) and unprocessed (fresh) spinach samples, they used RPHPLC to do the quantification. They used benzene to extract the material. They discovered that while the processed sample had dimethoate residue, the untreated spinach had a little amount that was slightly above the tolerance level. In comparison to the unprocessed samples, the washed samples (three times under regular tap water) displayed a 28–50% decrease, while the cooked samples (boiling for 15 minutes) displayed a 58–71% reduction. Greens should be washed three times with regular water before being cooked for fifteen minutes to greatly reduce the pesticide residue and bring it far under the Environmental Protection Agency's tolerance level (EPA).

Liu et al. (2014) determined four pesticide residues (phoxim, chlorpyrifos, imidacloprid and chlorantraniliprole) in bamboo shoot using quick, easy, cheap, effective, rugged and safe (QuEChERS)-matrix solid phase dispersion (MSPD) cleanup and liquid chromatography/mass spectrometry (LC-MS/MS). 5.0 g of the

sample were used, and 20 mL of acetonitrile was used for extraction and 2.0 g for cleanup (PSA). The outcomes demonstrated how easy, quick, and efficient the new QuEChERS-MSPD-LC-MS/MS technology is. At two concentration levels (20 and 200 g/kg), average recoveries were between 87.5 and 107.2%, with RSD values ranging from 5.2 to 12.4%, and detection limit (LOD) was obtained below the regulatory maximum residual limits for the pesticides. Sample preparation takes only 30 minutes, which is quicker than using the conventional 33 standard procedure. (at least 400 min). Therefore, the newly discovered approach is more environmentally friendly because it uses less solvent.

He et al. (2016) investigated the concentrations and dissipation of difenoconazole and fluxapyroxad residues in apples and soil using ultrahigh-performance liquid chromatography electrospray ionization tandem mass spectrometry (UHPLC-ESI-MS/MS). The 11.7 % SC was sprayed onto apple trees and soil in Beijing, Shandong, and Anhui provinces, China, at an application rate of 118 g a.i. ha⁻¹. After that, the dissipation of difenoconazole was monitored. UHPLC-ESI-MS/MS was used to determine residual difenoconazole concentrations. The difenoconazole half-lives in apples and soil were 6.2–9.5 and 21.0–27.7 days, respectively. Difenoconazole residue in apples and soil after the 11.7 % SC had been sprayed twice and three times, with 10 days between applications, at 78 g a.i. ha⁻¹ were measured. Representative apple and soil samples were collected after the last treatment, at preharvest intervals of 14, 21, and 28 days. The difenoconazole residue concentrations in apples and soil were 0.002–0.052 and 0.002–0.298 mg/kg, respectively. The difenoconazole residue concentrations in apples were lower than the maximum residue limits (0.5 mg/kg). An

application rate of 78 g a.i. ha⁻¹ is therefore recommended to ensure that treated apples can be considered safe for humans to consume.

Paramasivam (2022) conducted a research on persistence, dietary and ecological risk assessment of chlorantraniliprole in brinjal ecosystem using GC-MS. The average recoveries of chlorantraniliprole were in the range of 92.7–98.5% with a relative standard deviation of 1.1–3.3% and the matrix effect was less than 2%. Even on day the application of chlorantraniliprole consumer dietary risk assessment indicated that risk quotient was less than one and does not pose a risk to the health of consumer. The calculated environmental risk quotient (RQ) values were found to be less than one. This defines the low risk to earthworms and arthropods following the application chlorantraniliprole in the brinjal field.

Sheoran et al. (2023) conducted a field study to compare the efficacy of 10 recommended insecticides against *Earias* spp. on okra. Three okra plots (3 × 5 m²) were subjected to each of the 10 insecticidal sprays applied twice at an interval of 10 days. On the third, seventh, and tenth day after each spray, percent shoot and fruit infestation and yield were calculated. Shoot and fruit infestation reduced significantly by all insecticides compared to the control. Among them, chlorantraniliprole 18.5%SC was the most effective across all time points and resulted in the highest yield. A second field study, to determine the time till the maximum residue limit (MRL-0.3 mg/kg) and the limit of quantification (LOQ-0.01 mg/kg) for both fruit and soil exposed to either the recommended (125 mL/ha) or a double strength dose (250 mL/ha) of chlorantraniliprole 18.5% SC was undertaken. MRL was reached on the same day

following both doses. The LOQ was reached on the seventh and tenth day at recommended and a double strength dose, respectively. Chlorantraniliprole 18.5% SC gives effective control. However, *Earias* spp. resistance has been observed in other crops. Thus, constant monitoring in the field is needed to ensure its effectiveness.

Alam et al. (2022) conducted a research using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD) for detection of organophosphorus pesticide residues in brinjal collected from five local markets of Mymensingh district of Bangladesh. Among the 30 analyzed samples of eggplant, 5 samples (17% of the total number of samples) contained residues of chlorpyrifos, quinalphos, and dimethoate.

Kanda et al. (2012) carried out a research using Gas Chromatography on the extracts from soil, water and vegetable samples. The concentrations of pesticide residues in soil samples are lower than 20 $\mu\text{g}/\text{kg}$ of dry material. Contamination levels for water samples vary from 0.02 to 1.1 $\mu\text{g}/\text{L}$ of dry material with the highest levels for metalaxyl M (1.1 $\mu\text{g}/\text{L}$) and for dimethoate (1 $\mu\text{g}/\text{L}$). In vegetables, the concentrations measured are between 0.01 and 0.1 mg/kg of dry material. All these concentrations are affected by a positive factor of the maximum limits of residues.

Schreiber and Wittrig (2010) conducted a research on “Enhanced LC/MS for the Quantitation and Identification of Pesticide in Food Sample” where he collected a variety of fruit and vegetable samples including apple, banana, carrot, cucumber, curry powder grapes, grapefruit, hazelnut, lemon, nectarine, orange, pear, raisin, raspberry,

red pepper, salad, spinach and tomato from a supermarket and extracted using QuEChERS procedure. They injected the extracted sample into a liquid chromatography tandem mass spectrometry system where a total number of 12 pesticides were detected. They found 70-120% recovery for most of the pesticide with %CV<15%. They also found methamidophos 130 µg/kg, omithoate 42 µg/kg, thiamethoxam 48 µg/kg, dimethoate 54 µg/kg, clothianidin 14 µg/kg, imadacloprid 2.4 µg/kg, promamocarb 98 µg/kg, carbyl 499 µg/kg, metalaxy l 5.1 µg/kg, myclobutanil 3.4 µg/kg, spinosyn A 6.1 µg/kg, spinosyn D 6.8 µg/kg.

Malhat et al. (2021) investigated the dissipation behaviour and the consumer risk assessment of spitotetramat, flonicamid, imidacloprid and pymetrozine in open field strawberries. Insecticides were used in accordance with the recommended dosages and the stricter good agricultural practice (GAP) regimes. Depending on the drug, the initial concentrations ranged from 0.069 to 1.75 mg kg⁻¹, but the terminal residues, 14 days after the last administrations, and the dissipation half-lives were comparable. The half-lives for flonicamid and spitotetramat, imidacloprid, and pymetrozine were 2.8 days and 3.2 days, respectively, after application in accordance with the approved pattern. With exposure levels 2% of the acceptable daily intake (ADI) and 32% of the acute reference dose (ARfD) of each component, the dietary risk assessment, conducted using the hazard quotient and the EFSA PRIMo model, revealed no concern for consumer health.

Wang et al. (2022) carried out a research using modified QuEChERS and an optimized high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS)

for investigation on dynamics and influence of environmental factors on the dissipation of methoxyfenozide and pymetrozine residues in Chinese cabbage. The observed half-lives of methoxyfenozide and pymetrozine in cabbage samples ranged between two sampling seasons: in May–June, half-lives of methoxyfenozide and pymetrozine were 1.20 days and 1.89 days, respectively; during October–November, half-lives of methoxyfenozide and pymetrozine were 11.8 and 2.80 days, respectively. Meanwhile, a negative Spearman correlation was found between the residual concentrations and temperature ($p < 0.01$). This indicates that higher temperatures resulted in higher dissipation rates for methoxyfenozide and pymetrozine, suggesting that these pesticides degraded faster at higher temperatures. Additionally, higher pesticide residues in Chinese cabbage during low-temperature seasons resulted in higher risk quotients (RQ) ($RQ > 1$) for both analyzed compounds, which suggests that the effect of temperature on pesticide degradation needs to be considered as an essential factor while setting up the MRL.

Jia et al. (2019) carried out a research to investigate the occurrence of pymetrozine residues in cauliflower samples obtained from six cauliflower-producing areas of China during fixed time periods in 2017 and estimate the dietary risk of pymetrozine in cauliflower. A liquid chromatography with tandem mass spectrometry method was developed and validated to detect pymetrozine in cauliflower. The samples were extracted using 20 mL of acetonitrile and purified with dispersive solid-phase extraction using C_{18} as sorbent. The limit of quantification of pymetrozine was 0.008 mg/kg in cauliflower. The recoveries of the analyte were 82.04–95.18% with $RSD < 8.45\%$. The calibration curves for pymetrozine showed good linearities in the concentration range 0.004–2.0 mg/L with determination coefficients (R^2) > 0.999 .

Pymetrozine dissipated rapidly in cauliflower with a half-life of <4 days. The terminal residues of pymetrozine were <0.008–0.0881 mg/kg in cauliflower at 7, 10 and 14 days after spraying from six sites. The routine washing process removed about half amount of the pymetrozine in cauliflower; the reduction ratios were 51.0–52.8%. The dietary risk assessment indicated that pymetrozine did not exhibit obvious dietary health risks in cauliflower when good agricultural practices were implemented.

CHAPTER 3

MATERIALS AND METHODS

3.1. Reagents and Chemicals

All required pesticide standards (difenoconazole, dimethoate, pymetrozine, chlorantraniliprole) were supplied by Sigma-Aldrich Laborchemikalien (St Louis, MO, USA) via Kuri and Company Pvt. Ltd. HPLC grade acetonitrile (ACN), analytical-grade sodium chloride (NaCl), anhydrous magnesium sulphate (MgSO₄), ammonium acetate (NH₄OAc), and 99.98% formic acid were used. Primary secondary amine (PSA), graphitised carbon black (GCB) and C₁₈ were used as dispersive adsorbent. Before using water was collected from a Milli-Q apparatus through purification.

3.2. Preparation of standard in solvent

100 mg/L stock standard solutions of DFN, DMT, PYM and CLP were prepared separately using ACN as solvent. From stock solutions individual intermediate standard solutions (10 mg/L) was prepared with further dilution with ACN. Again mixing above four intermediate standard solutions one working standard solution (2 mg/L) was prepared with the same solvent. Finally, from the working standard solution 0.2 mg/L, 0.1 mg/L, 0.05 mg/L, 0.02 mg/L, 0.01 mg/L, 0.005 mg/L 0.002 mg/L and 0.001 mg/L were prepared by serial dilution. All the standard stock solutions and above working solutions were preserved at -20 °C.

3.3. Preparation of standard in sample matrix

Similar working standard solution 0.2 mg/L, 0.1 mg/L, 0.05 mg/L, 0.02 mg/L, 0.01 mg/L, 0.005 mg/L 0.002 mg/L and 0.001 mg/L were made by using extract of control sample as the previous standards prepared in acetonitrile. All standard solutions were stored in a refrigerator at -20°C before usage.

3.4. Sampling and sample preservation

100 brinjal samples were collected altogether for this investigation. Samples were collected from direct field level of Jessore region and some from retail markets of Dhaka during November 2021. Each collected sample amount was around 1kg and these were labeled properly in separate transparent zipper bags to protect from contamination. Samples were kept in ice box for temporary preservation immediately after collection. Collected samples were carried properly to INARS, BCSIR, Bangladesh. Then all the samples were finely chopped, blended and kept properly in labeled zipper bags to store these at -20°C for further analysis.

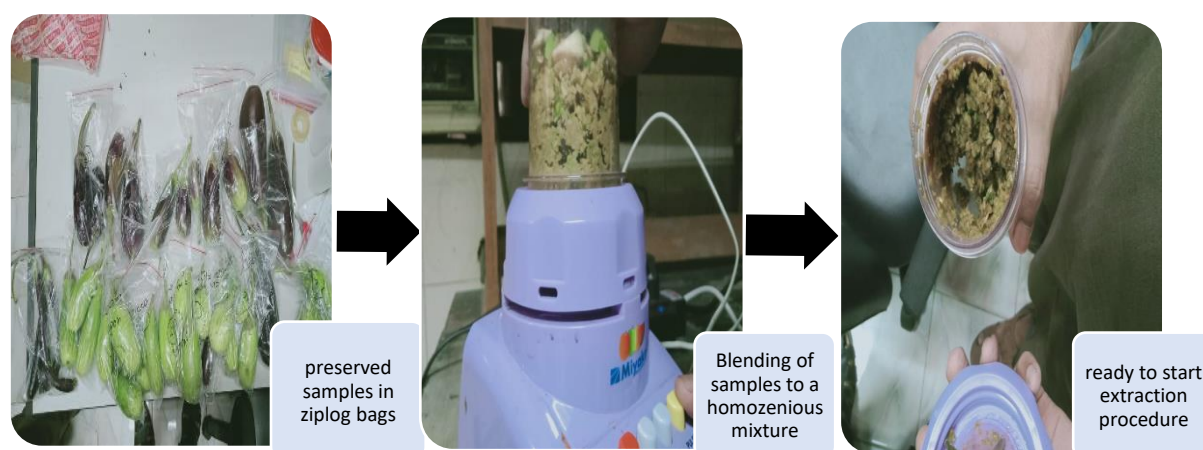


Figure. 2. Sample preparation for extraction.

Table 1. Sources and places of collection of brinjal samples

Area of collection			Sample ID
Directly from field	Jessore Sadar Upazila	Khajura Bazar	F1
			F2
			F3
			F4
			F5
			F6
			F7
			F8
			F9
			F10
			F11
			F12
			F13
			F14
			F15
		Satmail Bazar	F16
			F17
			F18
			F19
			F20
			F21
			F22
			F23
			F24
			F25
			F26
			F27
			F27
			F29
			F30
	Bagherpara Upazila	Sabaspur Village	F31
			F32
			F33
			F4
			F35
			F36
			F37
			F38
			F39

Area of collection			Sample ID
		Jahurpur Village	F40
			F41
			F42
			F43
			F44
			F45
			F46
			F47
			F48
			F49
			F50
From Market	Mirpur-1 Kacha Bazar	M1	
		M2	
		M3	
		M4	
		M5	
		M6	
		M7	
	Mirpur-6 Kacha Bazar	M8	
		M9	
		M10	
		M11	
		M12	
		M13	
		M14	
	Kawran Bazar Kacha Bazar	M15	
		M16	
		M17	
		M18	
		M19	
		M20	
		M21	
	Malibagh Kacha Bazar	M22	
		M23	
		M24	
		M25	
		M26	
		M27	
		M28	
		M29	
		M30	

Area of collection		Sample ID
	Hatirpool Kacha Bazar	M31
		M32
		M33
		M34
		M35
	Noyabazar Kacha Bazar	M36
		M37
		M38
		M39
		M40
		M41
	Newmarket Kacha Bazar	M42
		M43
		M44
		M45
M46		
M47		
M48		
M49		
M50		

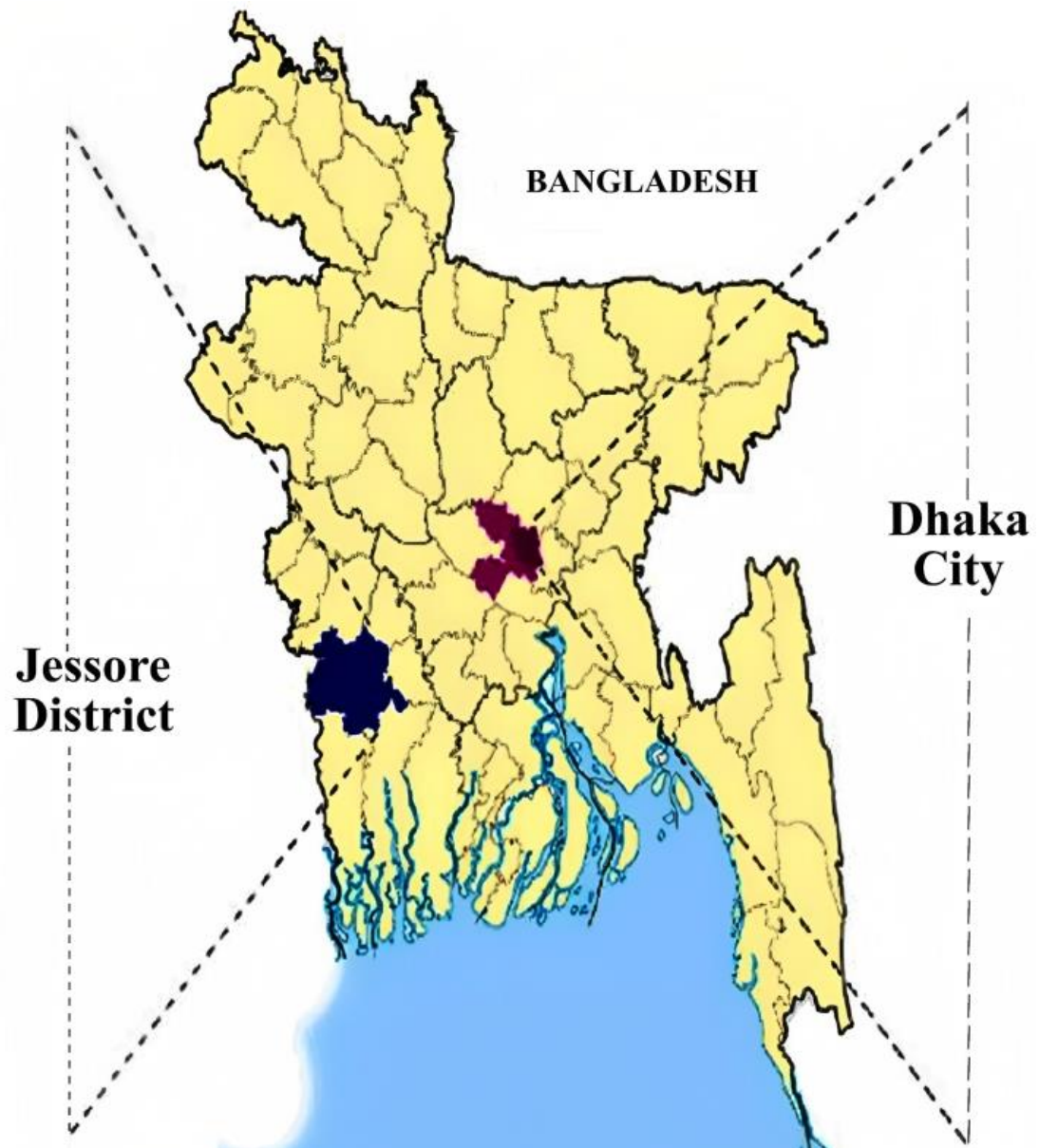


Figure 3. Map showing the location of Bangladesh from where the brinjal samples were collected.

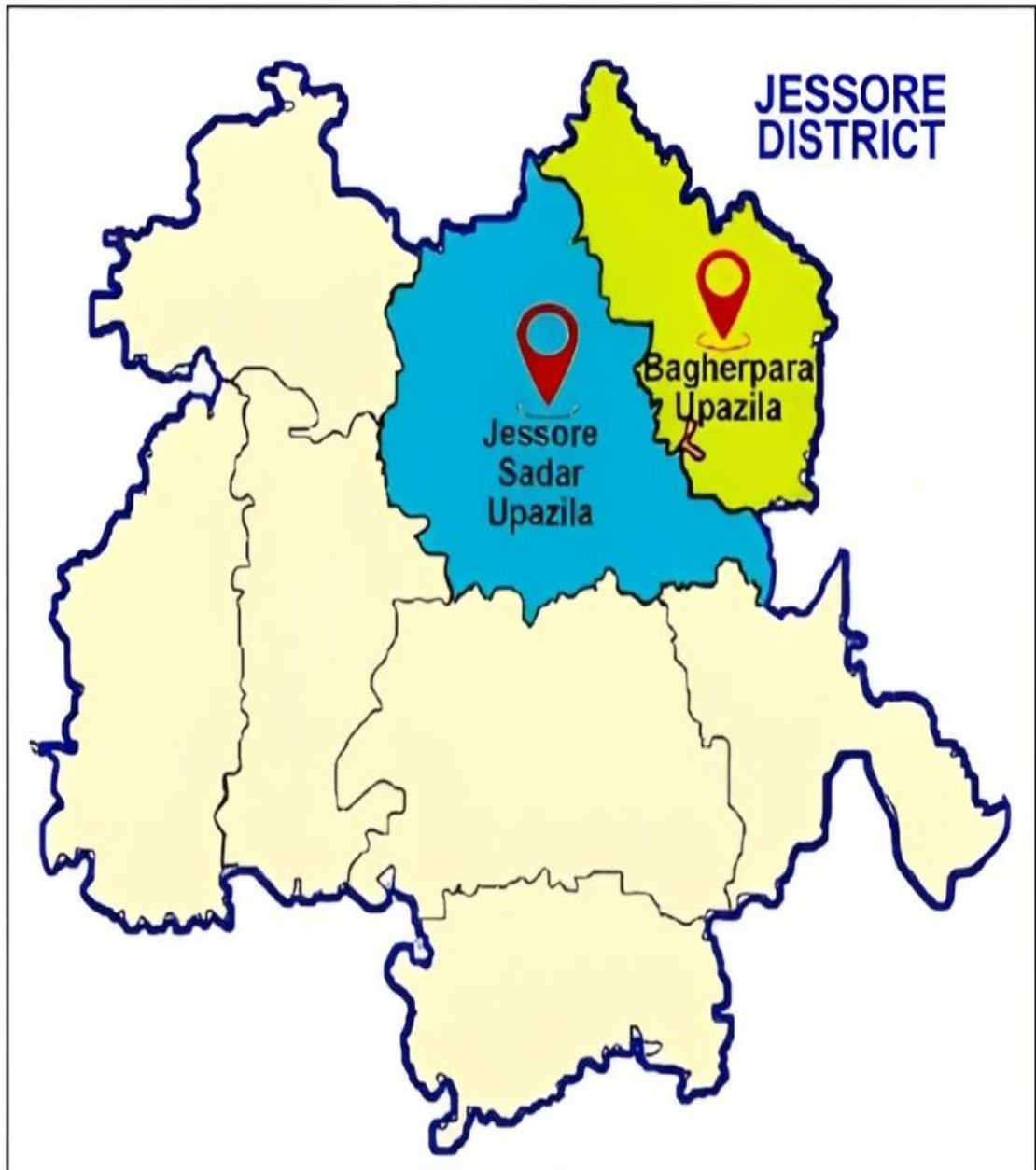


Figure 4. Map showing the location of Jessore district, Bangladesh from where the brinjal samples were collected.



Figure 5. Map showing the location of Dhaka city, Bangladesh from where the brinjal samples were collected.

3.5. Instrument

LC-MS/MS was used to carry out chromatographic analysis with an Agilent 1920 LC system coupled to a triple quadrupole system. The chromatographic separation were carried out through C18 column (2.1×100 mm, $1.8 \mu\text{m}$ particle size) with a flow rate of $0.45\text{mL}/\text{min}$. The column oven is maintained at 30°C temperature and the injection volume was $5\mu\text{L}$. The solvent system consisted of 50% (A): ACN and 50% (B): 0.1% formic acid + $10\text{mmol}/\text{L}$ ammonium acetate in water. The retention times of PYM, DMT, DFN and CLN were around 77.08 sec, 113.2 sec, 319.3 sec and 1036.6 sec, respectively, with a total run time of 25 mins. Positive electrospray ionization (ESI+) interface with triple quadrupole LC-MS/MS (QQQ) in multiple reaction monitoring (MRM) mode was conducted with two mass transitions where both of the target analytes yielded $[\text{M}+\text{H}]^+$ precursor ions. For quantitation, the most intense peak of the two mass transitions was used, while another was used for confirmation (Table 2).

PYM precursor ion with 218.1 molecular weight divided into product ions of 105 and 79 molecular weight. Here product ion with 105 molecular weight was used as quantifier and product ion with 79 molecular weight was used as qualifier.

DMT precursor ion with 230 molecular weight divided into product ions of 125 and 199 molecular weight. Here product ion with 125 molecular weight was used as quantifier and product ion with 199 molecular weight was used as qualifier.

DFN precursor ion with 406 molecular weight divided into product ions of 251 and 337 molecular weight. Here product ion with 251 molecular weight was used as quantifier and product ion with 337 molecular weight was used as qualifier.

Table 2. Fragmentation pattern of four pesticides in MRM mode

Pesticides name	Precursor ion, (M+H⁺)	Product ions	Collision energy, eV	Dwell energy, eV	Fragmentation energy, eV
Pymetrozine	218.1	105 <i>a</i>	20	200	135
		79 <i>b</i>	20	200	135
Dimethoate	230	125 <i>a</i>	29	200	135
		199 <i>b</i>	13	200	135
Difenoconazole	406	251 <i>a</i>	30	200	135
		337 <i>b</i>	30	200	135
Chlorantraniliprole	484.2	453.1 <i>a</i>	11	200	135
		286.1 <i>b</i>	11	200	135

N. B: *a* = Quantifier, *b* = Qualifier

CLN precursor ion with 484.2 molecular weight divided into product ions of 453.1 and 286.1 molecular weight. Here product ion with 453.1 molecular weight was used as quantifier and product ion with 286.1 molecular weight was used as qualifier.

3.6. Preparation of calibration curve

Working standard solution 0.2 mg/L, 0.1 mg/L, 0.05 mg/L, 0.02 mg/L, 0.01 mg/L, 0.005 mg/L 0.002 mg/L and 0.001 mg/L were vial up and sent for LC-MS/MS run. Peaks area for the pesticides were recorded. From the recorded peaks of clorantraniliprole a calibration curve was made. R² value for clorantraniliprole was 0.9995.

Table 3. Peak area in LC-MS/MS run for clorantraniliprole

Chlorantraniliprole	Lowest (ppm)	Injection Volume ($\mu\ell$)
	0.001	5
Ppm	ng	Area
0.2	1	48281
0.1	0.5	23125
0.05	0.25	11850
0.02	0.1	4340
0.01	0.05	1983
0.005	0.025	1206
0.002	0.01	571
0.001	0.005	388

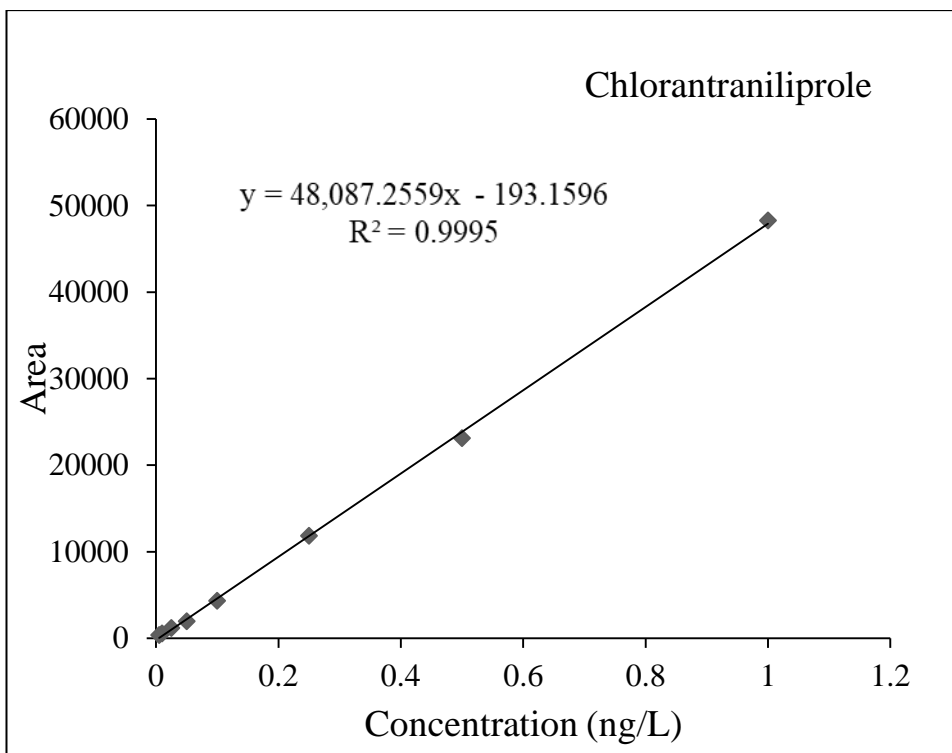


Figure 6. LC-MS/MS calibration curve for clorantraniliprole

Similarly, from the recorded peaks of difenoconazole a calibration curve was made.

R^2 value for difenoconazole was 0.9987.

Table 4. Peak area in LC-MS/MS run for difenoconazole

Difenoconazole	Lowest (ppm)	Injection Volume ($\mu\ell$)
		0.001
ppm	ng	Area
0.2	1	125804
0.1	0.5	58295
0.05	0.25	29148
0.02	0.1	11140
0.01	0.05	5683
0.005	0.025	2799
0.002	0.01	1219
0.001	0.005	432

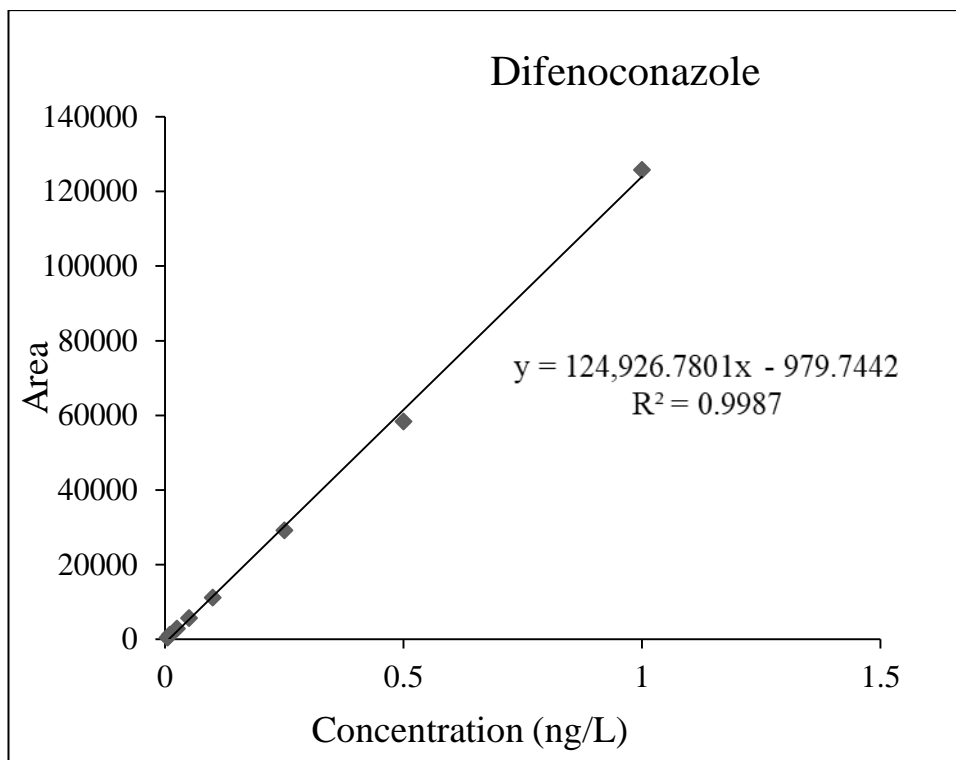


Figure 7. LC-MS/MS calibration curve for difenoconazole

As well as, from the recorded peaks of dimethoate a calibration curve was made. R² value for dimethoate was 0.9964.

Table 5. Peak area in LC-MS/MS run for dimethoate

Dimethoate	Lowest (ppm)	Injection Volume ($\mu\ell$)
	0.001	5
ppm	ng	Area
0.2	1	31182
0.1	0.5	13783
0.05	0.25	6712
0.02	0.1	2756
0.01	0.05	1184
0.005	0.025	674
0.002	0.01	317
0.001	0.005	200

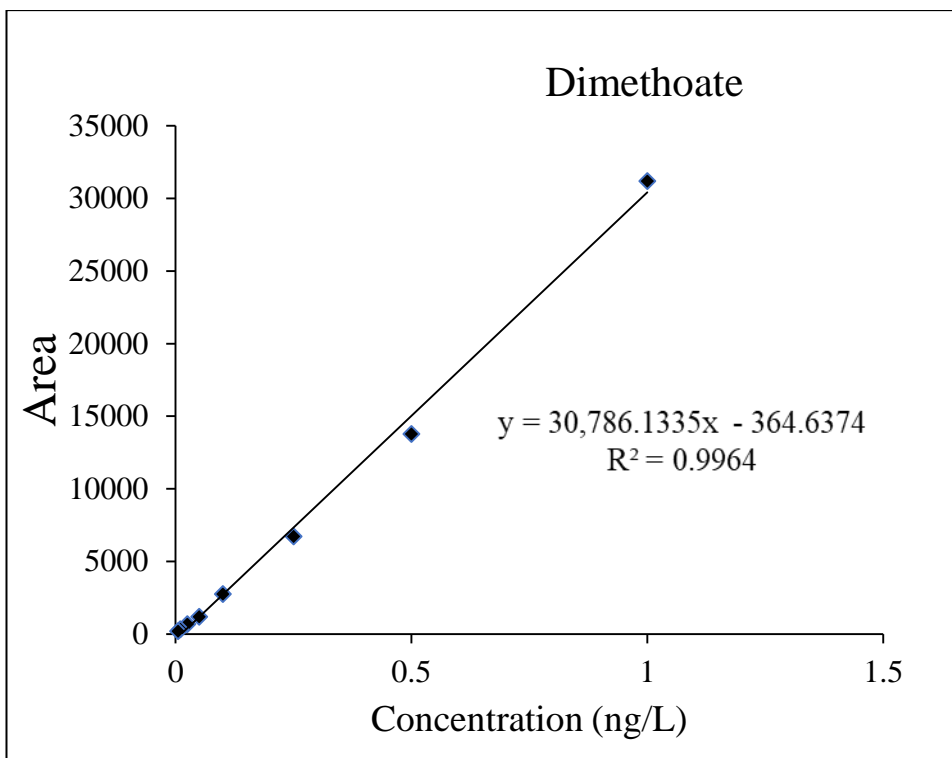


Figure 8. LC-MS/MS calibration curve for dimethoate

And lastly, as well as, from the recorded peaks of pymetrozine a calibration curve was made. R^2 value for pymetrozine was 0.9992.

Table 6. Peak area in LC-MS/MS run for pymetrozine

Pymetrozine	Lowest (ppm)	Injection Volume ($\mu\ell$)
		0.001
mg/L	ng	Area
0.2	1	120487
0.1	0.5	55196
0.05	0.25	27115
0.02	0.1	10717
0.01	0.05	5418
0.005	0.025	2358
0.002	0.01	1091
0.001	0.005	493

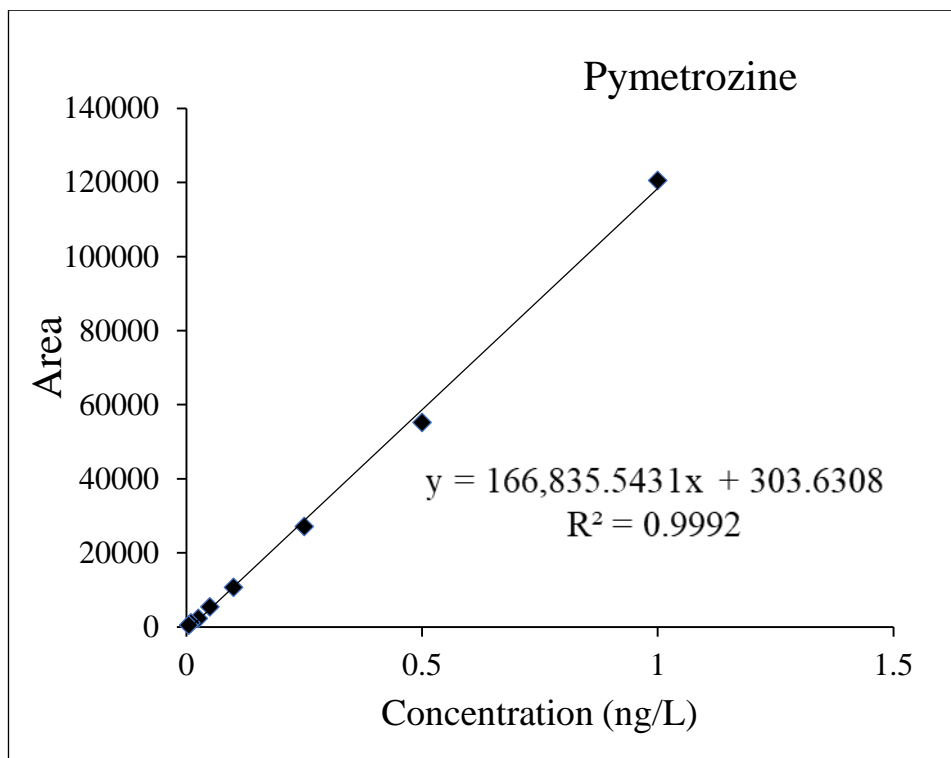


Figure 9. LC-MS/MS calibration curve for pymetrozine

3.7. Determination of cleanup material amount

To determine the appropriate amount of cleanup chemical such as PSA, GCB and C₁₈ to be used an LC-MS/MS experiment was carried out.

From previously prepared working standard solution (0.2 mg/L), 1.5 ml was transferred to several 2 ml centrifuge tubes containing 20 mg PSA, 30 mg PSA, 40 mg PSA, 50 mg PSA, 60 mg PSA, 70 mg PSA, 2 mg C₁₈, 3 mg C₁₈, 5 mg C₁₈, 10 mg C₁₈, 15 mg C₁₈, 20 mg C₁₈, 2 mg GCB, 5 mg GCB, 7 mg GCB, 10 mg GCB separately. Then the tubes were vortexed for 1 minute. After that supernatant from each tubes were filtered separately using 0.45 μm syringe filter and taken in 2 ml vials. A vial was filled with 0.2 mg/L standard solution. The vials were sent to LC-MS/MS to determine the perfect amount of absorbent.

Table 7. Area of peak with different amount of cleanup materials

Concentration	Pymetrozine	Dimethoate	Difenoconazole	Chlorantraniliprole
0.2 mg/L	1832 64200	12488 4579	26901	10066 11679
0.2 mg/L+ 20 mg PSA	1859 61863	10212 3759	28108	9747 12014
0.2 mg/L+ 30 mg PSA	1837 61825	10446 3719	29317	10040 12044
0.2 mg/L+ 40 mg PSA	1812 560502	10430 3556	28863	9571 11959
0.2 mg/L+ 50 mg PSA	1788 60912	11852 4268	29772	9941 12013
0.2 mg/L+ 60 mg PSA	1634 58736	12762 4628	27972	9608 11924
0.2 mg/L+ 70 mg PSA	1581 55602	12542 4519	27956	9250 11476
0.2 mg/L+ 2 mg C₁₈	2032 71917	6729 4969	29742	9495 11844
0.2 mg/L+ 3 mg C₁₈	1933 70975	13676 4663	29053	9141 10627
0.2 mg/L+ 5 mg C₁₈	1730 60663	6063 4096	27795	9482 11607
0.2 mg/L+ 10 mg C₁₈	1662 58958	5967 3957	27662	9362 11400
0.2 mg/L+ 15 mg C₁₈	1756 59266	5740 4375	28643	9214 11473
0.2 mg/L+ 20 mg C₁₈	1707 60347	5747 2260	30141	9550 11504
0.2 mg/L+ 2 mg GCB	1893 60785	6321 2312	30002	8886 10475
0.2 mg/L+ 5 mg GCB	1778 60606	5519 3866	27568	9501 11694
0.2 mg/L+ 7 mg GCB	1406 47604	6439 2609	29981	6664 8339
0.2 mg/L+ 10 mg GCB	1184 41290	7432 5496	29437	6004 7240

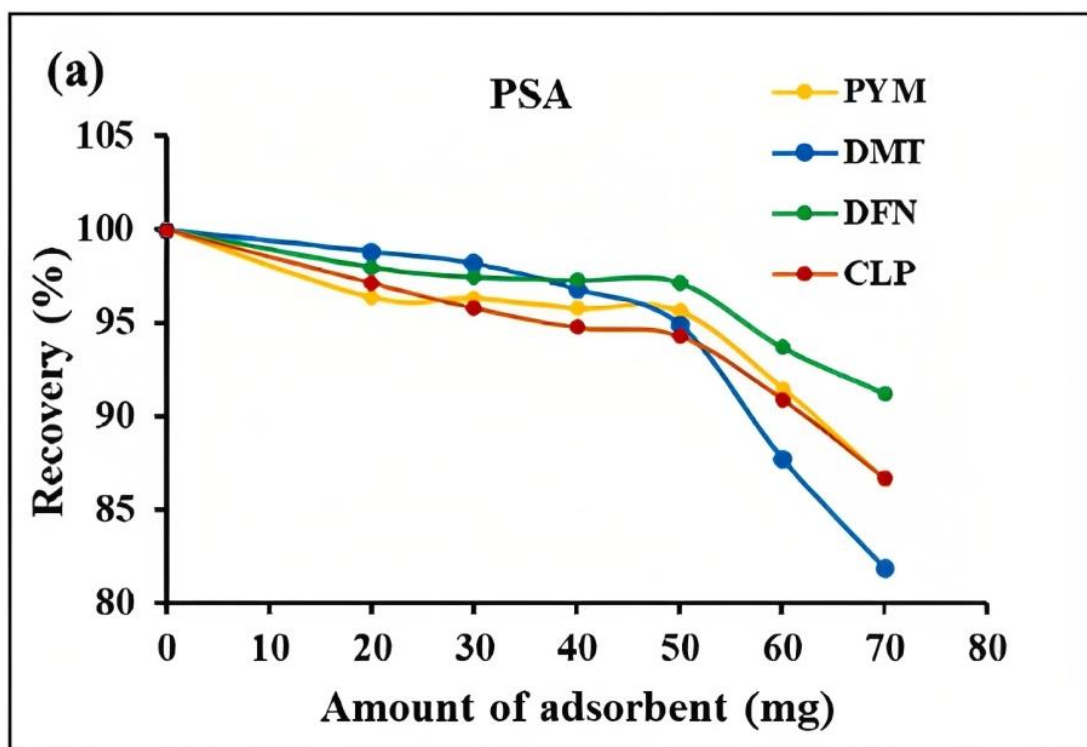


Figure 10. Recovery optimization of PSA amounts.

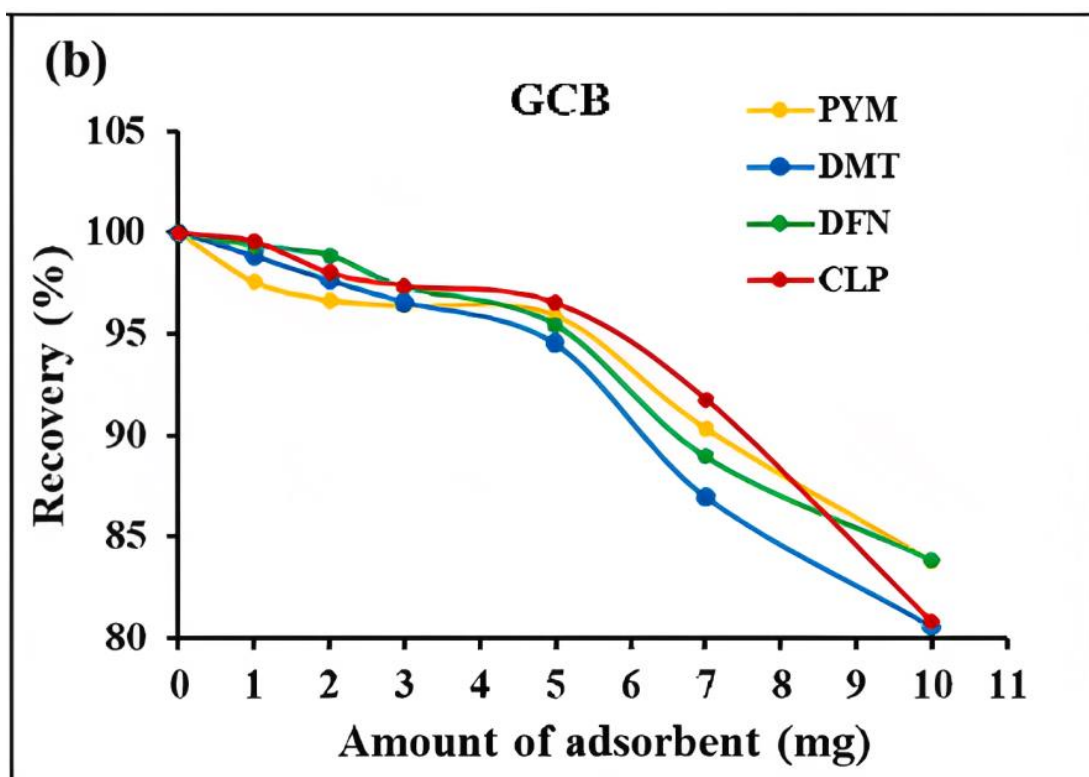


Figure 11. Recovery optimization of GCB amounts.

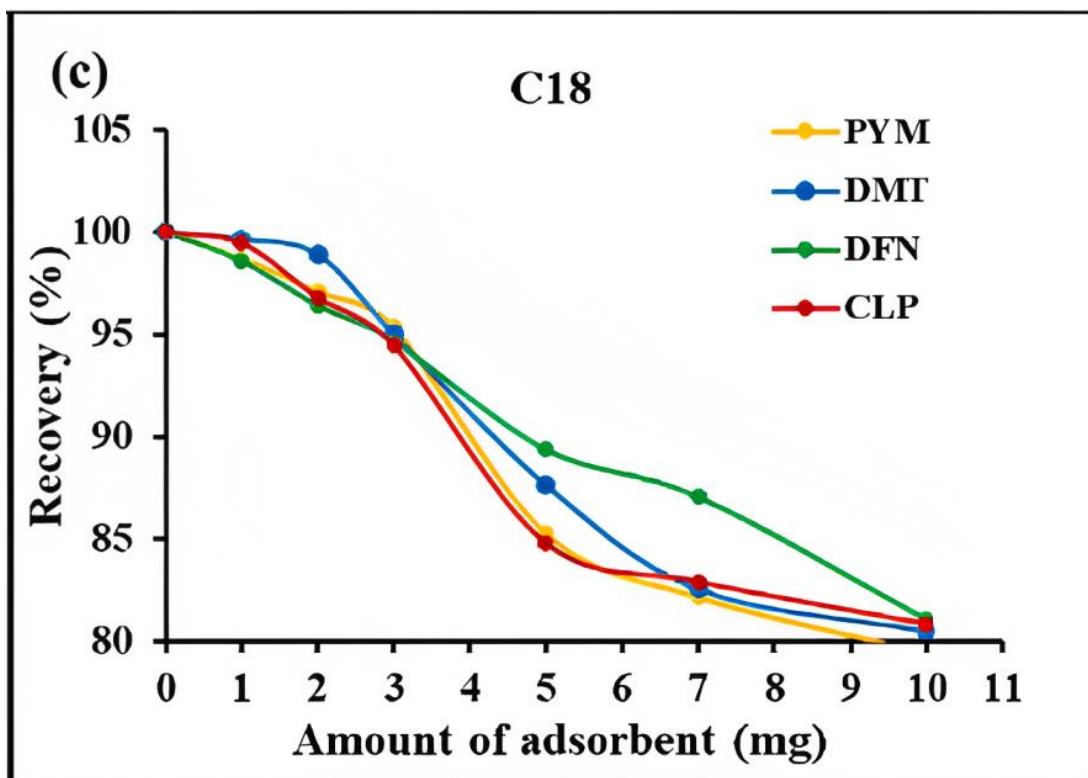


Figure 12. Recovery optimization of C₁₈ amounts.

From the results, 50 mg PSA, 3 mg C₁₈ and 5 mg GCB was selected as appropriate cleanup materials. Because we got minimum of 95% recovery for all the analytes at 50 mg PSA (Figure 8). Similarly for GCB, 1–10 mg (1, 2, 3, 5, 7, and 10 mg), we got minimum of 90% recovery for all the analytes at 5 mg (Figure 9). Lastly, we analyzed the recovery for C₁₈, using 1–10 mg (1, 2, 3, 5, 7, and 10 mg), we got minimum of 95% recovery for all the analytes at 3 mg (Figure 10).

3.8. Extraction and cleanup

Modified QuEChERS method was applied for conducting sample preparation which include extraction as well as cleanup portion.

In terms of extraction, approximately 5 g of chopped and blended brinjal sample was weighed into 50-mL teflon centrifuge tubes. After that 10 mL of ACN was added into

sample followed by manual shaking. Then extraction salt was added to the sample (3 g of anhydrous MgSO₄ and 1 g NaCl) and mixed using vortex in high speed for 1 min. 1.5 mL clear extract was transferred into a 2 mL micro-centrifuge tube which was previously filled with sorbent (50 mg PSA, 3 mg C₁₈ and 5 mg GCB).

Then that micro-centrifuge tube was mixed with vortex for 30 sec and centrifuged at 4000 rpm for 5 min. At last the clean extracted supernatant was transferred through filtration with 0.45 µm microfilter to a vial for LC/MS/MS analysis.

CHAPTER 4

RESULTS AND DISCUSSIONS

The analysis was performed by LC-MS/MS. Data of residue obtained from LC-MS/MS was recorded and put in calibration curve to find out the residue concentration in samples. The concentration of the selected pesticides found from the injected extract of brinjal samples are shown in Table 8, Table 9, Table 10 and Table 11.

Table 8. Determination of pymetrozine residue in brinjal samples

Real sample	Peak area	Residual amount (ng)	Residual amount (ppm)	Concentration in the sample (ppm)	Average
F4	87,623	0.743	0.149	0.297	0.241
F13	15,608	0.140	0.028	0.056	
F29	67,004	0.570	0.114	0.228	
F42	112,724	0.953	0.191	0.381	
M28	4,552	0.048	0.010	0.019	0.137
M47	74,774	0.635	0.127	0.254	

Table 9. Determination of difenoconazole residue in brinjal samples

Real sample	Peak area	Residual amount (ng)	Residual amount (ppm)	Concentration in the sample (ppm)	Average
F4	85,232	0.723	0.145	0.289	0.195
F16	49,075	0.420	0.084	0.168	
F21	8,436	0.080	0.016	0.032	
F27	71,487	0.608	0.122	0.243	
F29	25,469	0.223	0.045	0.089	
F42	103,759	0.878	0.176	0.351	
M4	6,345	0.063	0.013	0.025	0.107
M28	78,360	0.665	0.133	0.266	
M31	26,365	0.230	0.046	0.092	
M47	12,022	0.110	0.022	0.044	

Table 10. Determination of dimethoate residue in brinjal samples

Real sample	Peak area	Residual amount (ng)	Residual amount (ppm)	Concentration in the sample (ppm)	Average
F4	70,291	0.598	0.120	0.239	0.186
F13	72,981	0.620	0.124	0.248	
F16	27,262	0.238	0.048	0.095	
F22	57,442	0.490	0.098	0.196	
F27	74,176	0.630	0.126	0.252	
F30	55,052	0.470	0.094	0.188	
F34	49,374	0.423	0.085	0.169	
F39	61,925	0.528	0.106	0.211	
F40	70,291	0.598	0.120	0.239	
F42	4,253	0.045	0.009	0.018	
M4	61,028	0.520	0.104	0.208	
M28	50,868	0.435	0.087	0.174	
M33	3,954	0.043	0.009	0.017	
M38	36,525	0.315	0.063	0.126	
M50	24,572	0.215	0.043	0.086	

Table 11. Determination of chlorantraniliprole residue in brinjal samples

Real sample	Peak area	Residual amount (ng)	Residual amount (ppm)	Concentration in the sample (ppm)	Average
F27	12,918	0.118	0.024	0.047	0.156
F13	69,993	0.595	0.119	0.238	
F18	6,046	0.060	0.012	0.024	
F23	31,146	0.270	0.054	0.108	
F30	56,247	0.480	0.096	0.192	
F39	75,371	0.640	0.128	0.256	
F50	67,004	0.570	0.114	0.228	
M4	3,058	0.035	0.007	0.014	0.198
M28	28,158	0.245	0.049	0.098	
M32	88,818	0.753	0.151	0.301	
M35	20,987	0.185	0.037	0.074	
M41	87,623	0.743	0.149	0.297	
M43	98,082	0.830	0.166	0.332	
M44	46,087	0.395	0.079	0.158	
M47	91,806	0.778	0.156	0.311	

4.1. Method validation

Linearity, specificity, accuracy, precision, limits of detection (LOD), limits of quantification (LOQ) all these were performed to validate the method. Correlation coefficient value (r^2) of eight-point calibration curve shows linearity. In terms of blank sample, there was not any peak at the same retention time of the target analyte which indicates the specificity of the method. From the recovery (%) accuracy of the result was ensured. Similarly the relative standard deviation, RSD (%) shows the precision. For analyte the response that gives signal-to-noise ratio (S/N) is 3 was expressed as LOD. Eventually the LOQ was estimated from the response of analyte which is ten times intense than the noise.

4.2. Method performance

4.2.1. Specificity

The specificity of the method was estimated by the analysis of blank brinjal sample in quintaples. Not any peak was found for sample matrix at the retention time of the target analytes. Therefore the current method of analysis is specific.

Chromatograms

Fragmentor Voltage	13	Collision Energy	0	Ionization Mode	ES
	5				I

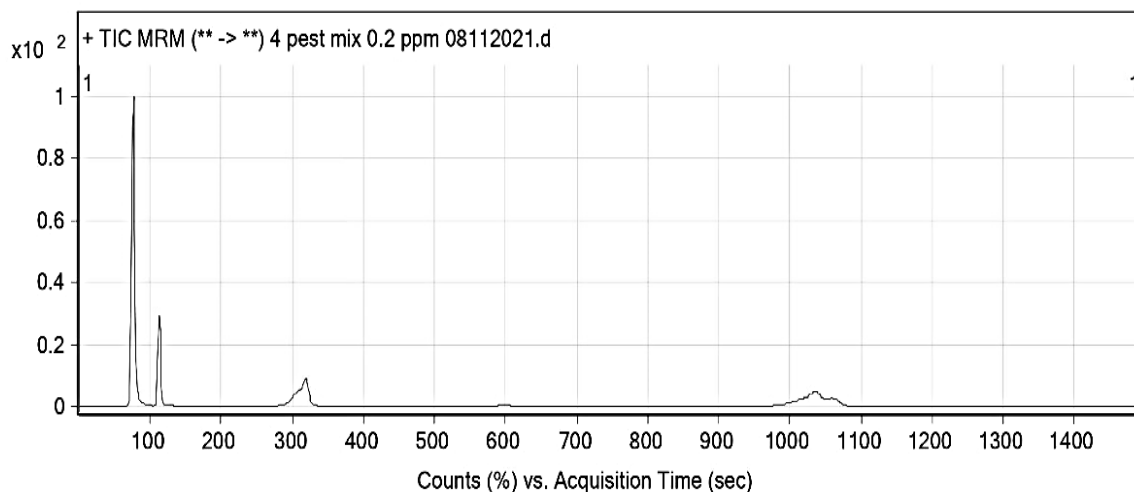


Figure 13. Total ion chromatogram of four mix pesticides of 0.2ppm concentration in LC-MS/MS.

4.2.2. Linearity

Eight-point calibration curve found from the working solution concentration range 0.001-0.2 mg/L gives correlation coefficient value (r^2) (Table. 12).

Similarly another eight point calibration curve was found from the working standard prepared in blank matrix. The correlation coefficient values (r^2) from matrix-matched calibration are (0.996-0.999).

Table 12. Recoveries, LODs and LOQs for individual pesticides in brinjal samples

Pesticides name	Linear range ($\mu\text{g/L}$) & linearity (R^2)	Recoveries [Mean \pm RSD] at different spiked levels (n=4)		LOD ($\mu\text{g/kg}$)	LOQ ($\mu\text{g/kg}$)
		Spiked level	Mean \pm RSD		
Pymetrozine	(0.5-200) 0.998	400 $\mu\text{g/kg}$	72.4 \pm 0.5	0.33	1.0
		200 $\mu\text{g/kg}$	70.3 \pm 4.7		
		20 $\mu\text{g/kg}$	70.9 \pm 5.2		
		8.0 $\mu\text{g/kg}$	107.2 \pm 6.3		
Dimethoate	(1.0-200) 0.996	400 $\mu\text{g/kg}$	82.5 \pm 5.3	0.66	2.0
		200 $\mu\text{g/kg}$	83.7 \pm 6.8		
		20 $\mu\text{g/kg}$	83.2 \pm 3.1		
		8.0 $\mu\text{g/kg}$	113.2 \pm 6.1		
Difenoconazole	(0.2-200) 0.998	400 $\mu\text{g/kg}$	78.5 \pm 6.7	0.15	0.4
		200 $\mu\text{g/kg}$	76.2 \pm 4.6		
		20 $\mu\text{g/kg}$	81.9 \pm 4.6		
		8.0 $\mu\text{g/kg}$	105.8 \pm 6.0		
Chlorantraniliprol e	(0.5-200) 0.999	400 $\mu\text{g/kg}$	76.7 \pm 2.2	0.33	1.0
		200 $\mu\text{g/kg}$	77.4 \pm 4.8		
		20 $\mu\text{g/kg}$	85.4 \pm 5.5		
		8.0 $\mu\text{g/kg}$	95.5 \pm 3.6		

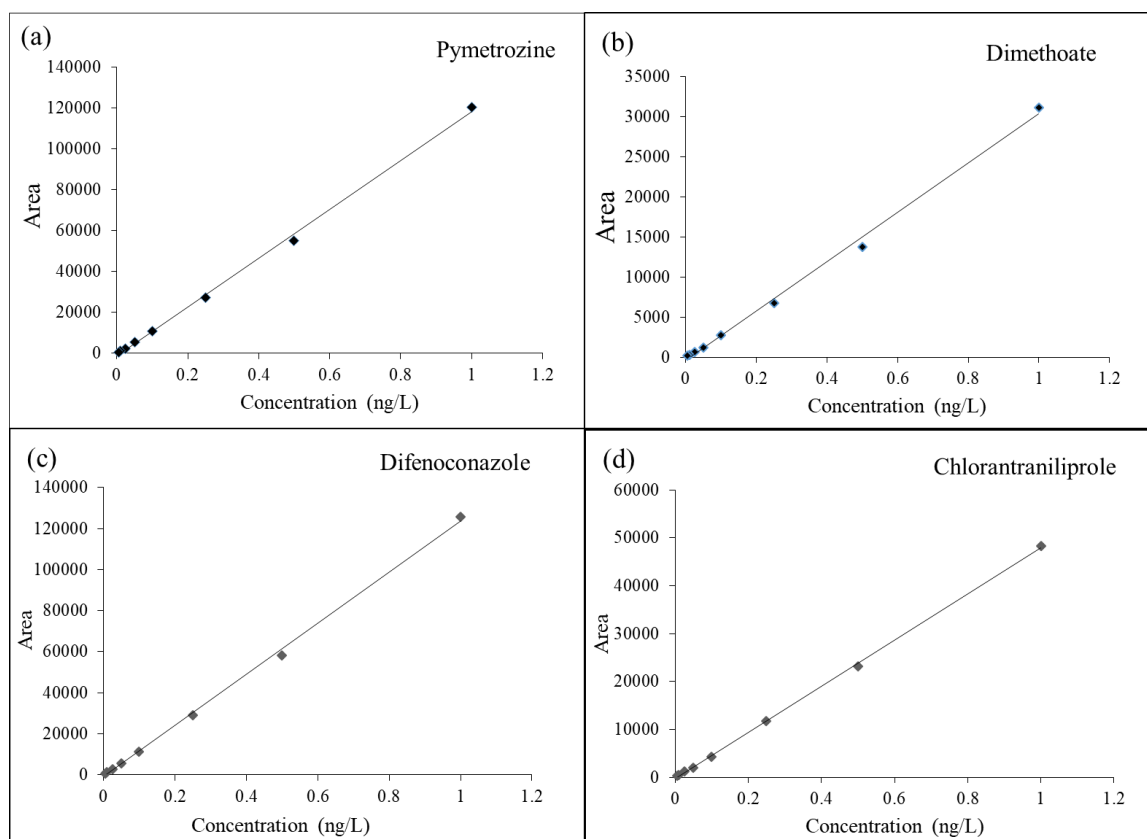


Figure 14. Four calibration curve of the selected pesticides

Table 13. Residue analysis data of four pesticides in brinjal samples

Vegetable	Sample source	Pesticide	No of sample		Detected samples %	MRL(mg/kg)	No of samples >MRL (mg/kg)	Residual range (mg/kg)	Mean residue (mg/kg)
			Analysed	Detected					
Brinjal	Field	Pymetrozine	50	4	8	0.01	4	0.056-0.381	0.241
		Dimethoate	50	10	20	0.01	10	0.018-0.252	0.186
		Difenoconazole	50	6	12	0.01	6	0.032-0.351	0.195
		Chlorantraniliprole	50	7	14	0.6	0	0.024-0.256	0.156
	Market	Pymetrozine	50	2	4	0.01	2	0.019-0.254	0.137
		Dimethoate	50	5	10	0.01	5	0.017-0.208	0.122
		Difenoconazole	50	4	8	0.01	4	0.025-0.266	0.107
		Chlorantraniliprole	50	8	16	0.6	0	0.014-0.332	0.198

4.2.3. LOD and LOQ

The values of LOD were (0.15-0.66) and LOQ were 0.4 to 2.0.

4.2.4. Recovery

Recovery studies were conducted (in quadruple) by spiking samples (5 g) with standard solutions. The average recoveries of pesticides levels of 400, 200, 20 and 8 $\mu\text{g}/\text{kg}$ were (76.90.20–105.42) %, with relative standard deviations (RSDs) of (0.5–6.8) % (Table. 13). Representative chromatograms are given in supplementary data.

Table 14. Recoveries, LODs and LOQs for individual pesticides in brinjal samples.

Pesticides name	Linear range ($\mu\text{g}/\text{L}$) and linearity (R^2)	Recovery at different spike levels		LOD ($\mu\text{g}/\text{kg}$)
		Spiked levels (n = 5) ($\mu\text{g}/\text{kg}$)	Recoveries [Mean \pm RSD] %	
PYM	0.5-200 (0.9982)	400	72.4 \pm 0.5	0.33
		200	70.3 \pm 4.7	
		100	78.5 \pm 2.5	
		20	70.9 \pm 5.2	
		8	107.2 \pm 6.3	
DMT	1.0-200 (0.9964)	400	82.5 \pm 5.3	0.66
		200	83.7 \pm 6.8	
		100	91.3 \pm 3.8	
		20	83.2 \pm 3.1	
		8	113.2 \pm 6.1	
DFN	0.2-200 (0.9987)	400	78.5 \pm 6.7	0.15
		200	76.2 \pm 4.6	
		100	87.9 \pm 2.3	
		20	81.9 \pm 4.6	
		8	105.8 \pm 6.0	
CLP	0.5-200 (0.9995)	400	76.7 \pm 2.2	0.33
		200	77.4 \pm 4.8	
		100	79.2 \pm 5.2	
		20	85.4 \pm 5.5	
		8	95.5 \pm 3.6	

4.3. Pesticides residue in brinjal

From the Table 13, we can see, in field samples, highest detected residue level of PYM was 0.381 and lowest detected residue level of PYM was 0.056. Whereas in market samples, highest detected residue level of PYM was 0.254 and lowest detected residue level of PYM was 0.019.

In field samples, highest detected residue level of DMT was 0.252 and lowest detected residue level of DMT was 0.018. Whereas in market samples, highest detected residue level of DMT was 0.208 and lowest detected residue level of DMT was 0.017.

In field samples, highest detected residue level of DFN was 0.351 and lowest detected residue level of DFN was 0.032. Whereas in market samples, highest detected residue level of DFN was 0.266 and lowest detected residue level of DFN was 0.025.

In field samples, highest detected residue level of CLP was 0.256 and lowest detected residue level of CLP was 0.024. Whereas in market samples, highest detected residue level of CLP was 0.332 and lowest detected residue level of CLP was 0.014.

Out of the fifty field samples four (8%) samples contain PYM, ten (20%) samples contain DMT, six (12%) samples contain DFN and seven (14%) samples contain CLP. However, in terms of market samples, out of fifty samples two (4%) samples contain PYM, five (10%) samples contain DMT, four (8%) samples contain DFN and eight (16%) samples contain CLP. The mean concentrations of detected pesticides residues were above their corresponding MRL levels except chlorantraniliprole.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The present research work was conducted to analysis the residues of difenoconazole, dimethoate, chlorantraniliprole, pymetrozine on brinjal fruits collected from different fields and markets of bangladesh at the Institute of National Analytical Research and Service (INARS) Laboratory, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka during July 2021 to December 2021. 100 samples collected from different places were studied. 50 samples collected from fields of Jashore. On the other hand, 50 samples collected from different markets of Dhaka. The level of difenoconazole, dimethoate, chlorantraniliprole, pymetrozine residues was detected using LC-MS/MS. A simple and practical LC-MS/MS method for the determination of the trace amounts of chlorantraniliprole, difenocaonazole, dimethoate, pymetrozine in brinjal was presented. Samples were extracted using ethanol and were cleaned up using PSA, GCB and C₁₈.

In field samples, the highest pymetrozine residue found was 0.381, the lowest was 0.056 and the mean was 0.241. Out of 50 field samples, pymetrozine was detected in 4 samples (8%) and all of them was above MRL. The highest dimethaote residue found was 0.252, the lowest was 0.018 and the mean was 0.186. Out of 50 field samples, dimethaote was detected in 10 samples (20%) and all of them was above MRL. The highest difenoconazole residue found was 0.351, the lowest was 0.032 and the mean was 0.195. Out of 50 field samples, difenoconazole was detected in 6 samples (12%) and all of them was above MRL. The highest chlorantraniliprole residue found was

0.256, the lowest was 0.024 and the mean was 0.195. Out of 50 field samples, chlorantraniliprole was detected in 7 samples (14%) and all of them was below MRL.

In market samples, the highest pymetrozine residue found was 0.254, the lowest was 0.019 and the mean was 0.137. Out of 50 market samples, pymetrozine was detected in 2 samples (4%) and all of them was above MRL. The highest dimethaote residue found was 0.208, the lowest was 0.017 and the mean was 0.122. Out of 50 market samples, dimethaote was detected in 5 samples (10%) and all of them was above MRL. The highest difenoconazole residue found was 0.266, the lowest was 0.025 and the mean was 0.107. Out of 50 market samples, difenoconazole was detected in 4 samples (8%) and all of them was above MRL. The highest chlorantraniliprole residue found was 0.332, the lowest was 0.014 and the mean was 0.198. Out of 50 market samples, chlorantraniliprole was detected in 8 samples (16%) and all of them was below MRL.

The result shows the occurrence of residue in brinjal samples more than MRL. This may be due to higher dose application of this pesticides more than recommended dose. The residue level is lower in market samples than in field samples. This must be due to dessociation of pesticides during transportation period. More regulation and awareness on proper application of recommended dose of pestice before 4-7 days of harvesting is required.

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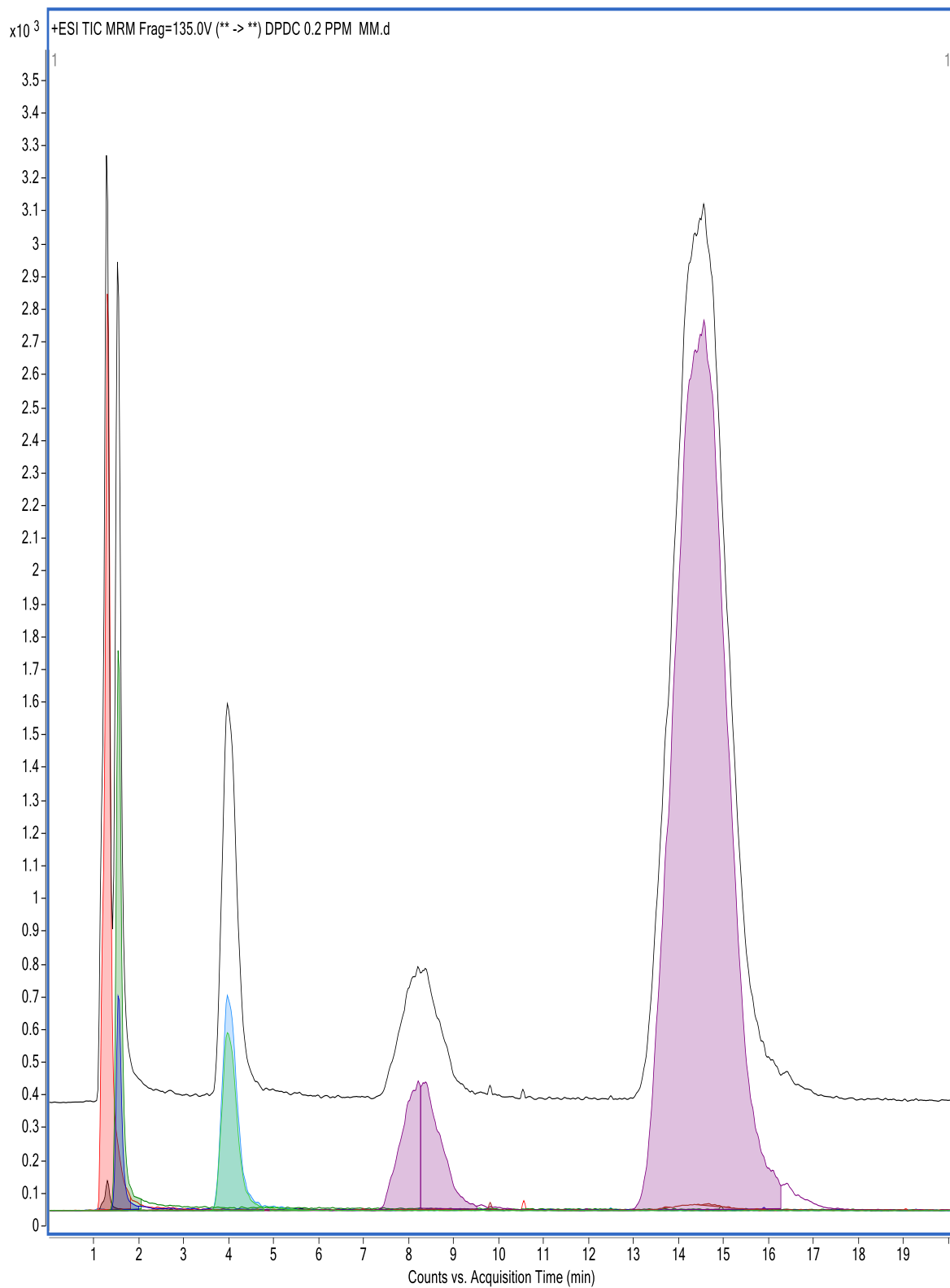


Figure 15: Chromatogram of recovery standard 0.2 ppm MM

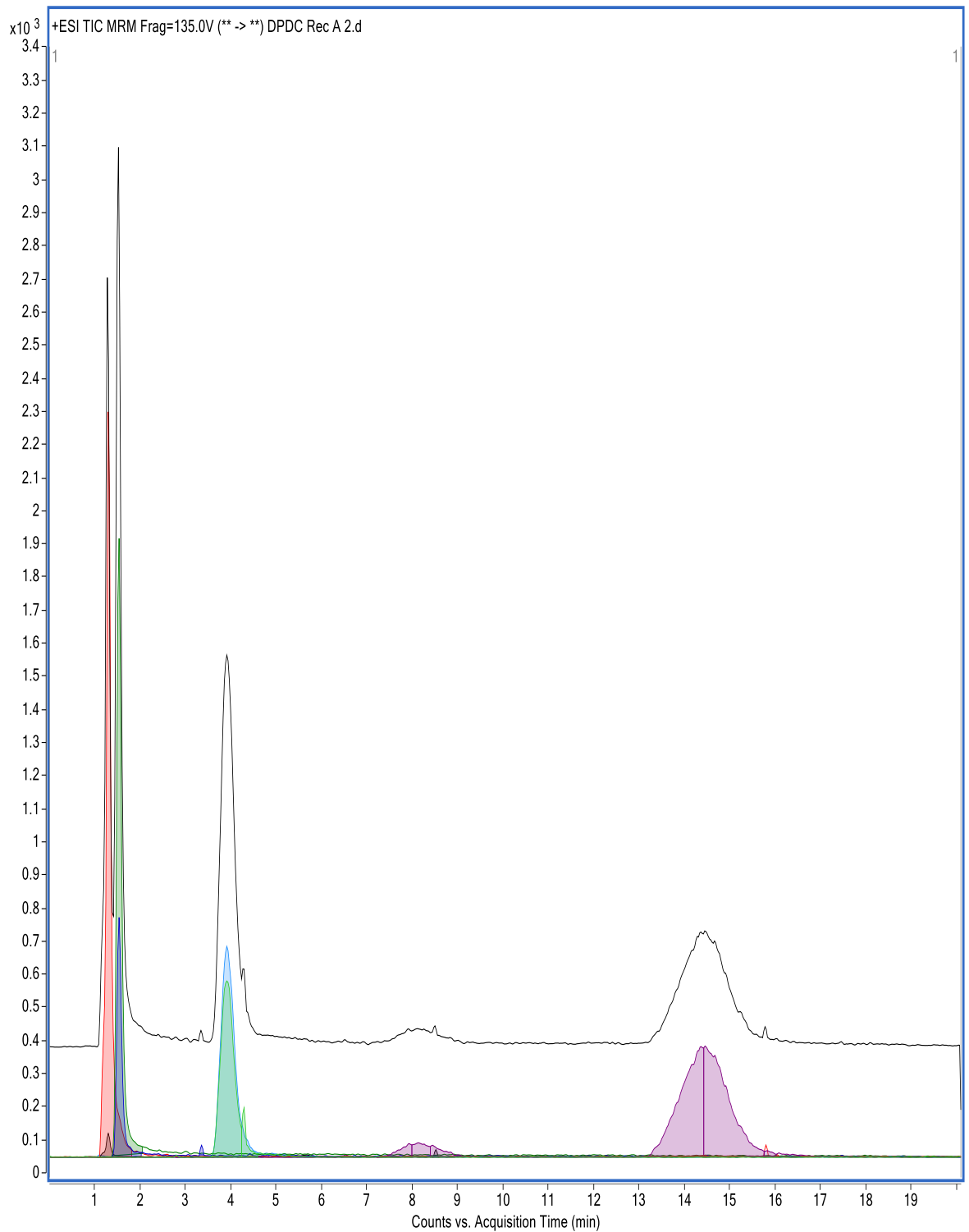


Figure 16: Chromatogram of recovery A2 (400 $\mu\text{g}/\text{kg}$ spiked level)

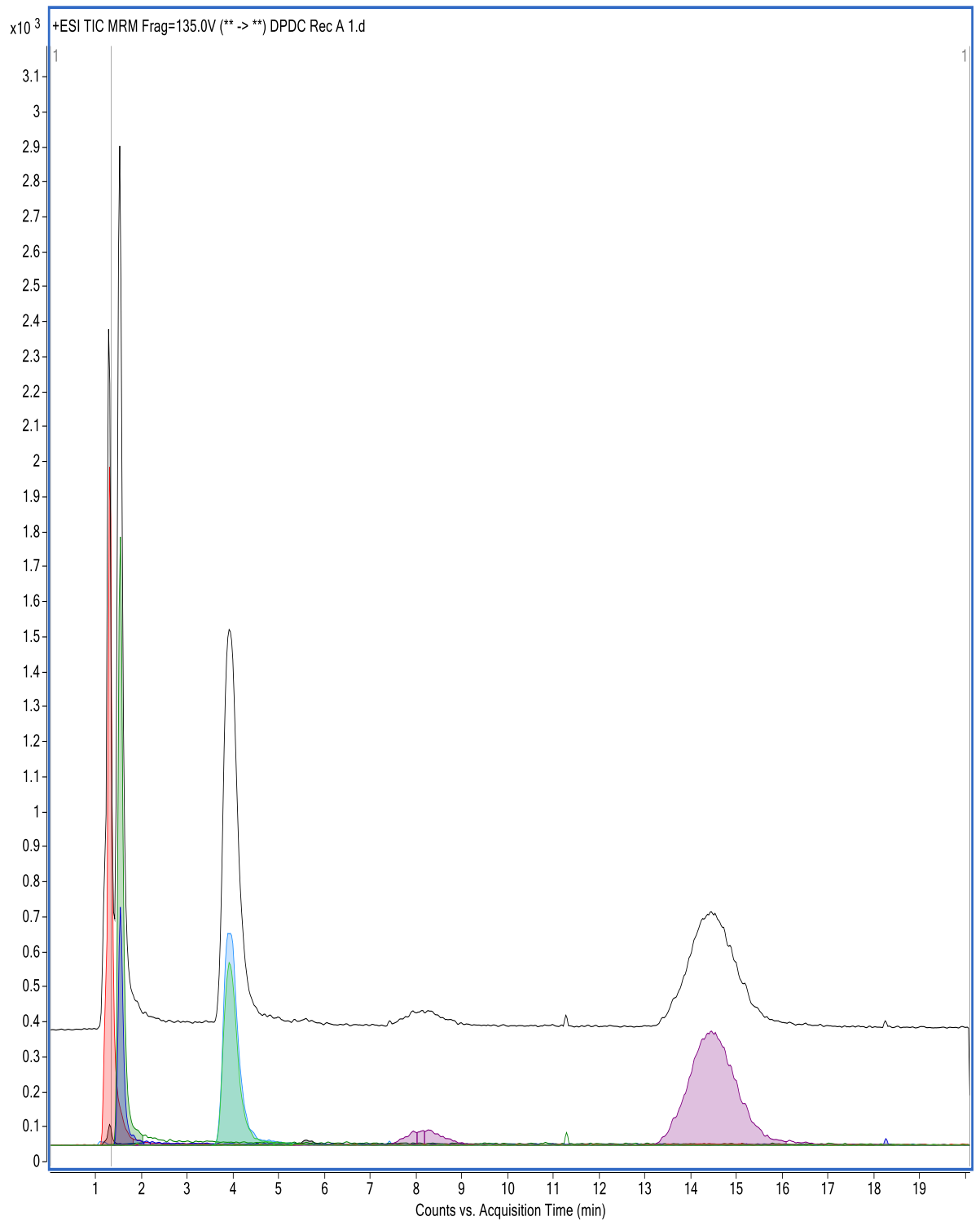


Figure 17: Chromatogram of recovery A1 (400 µg/kg spiked level)

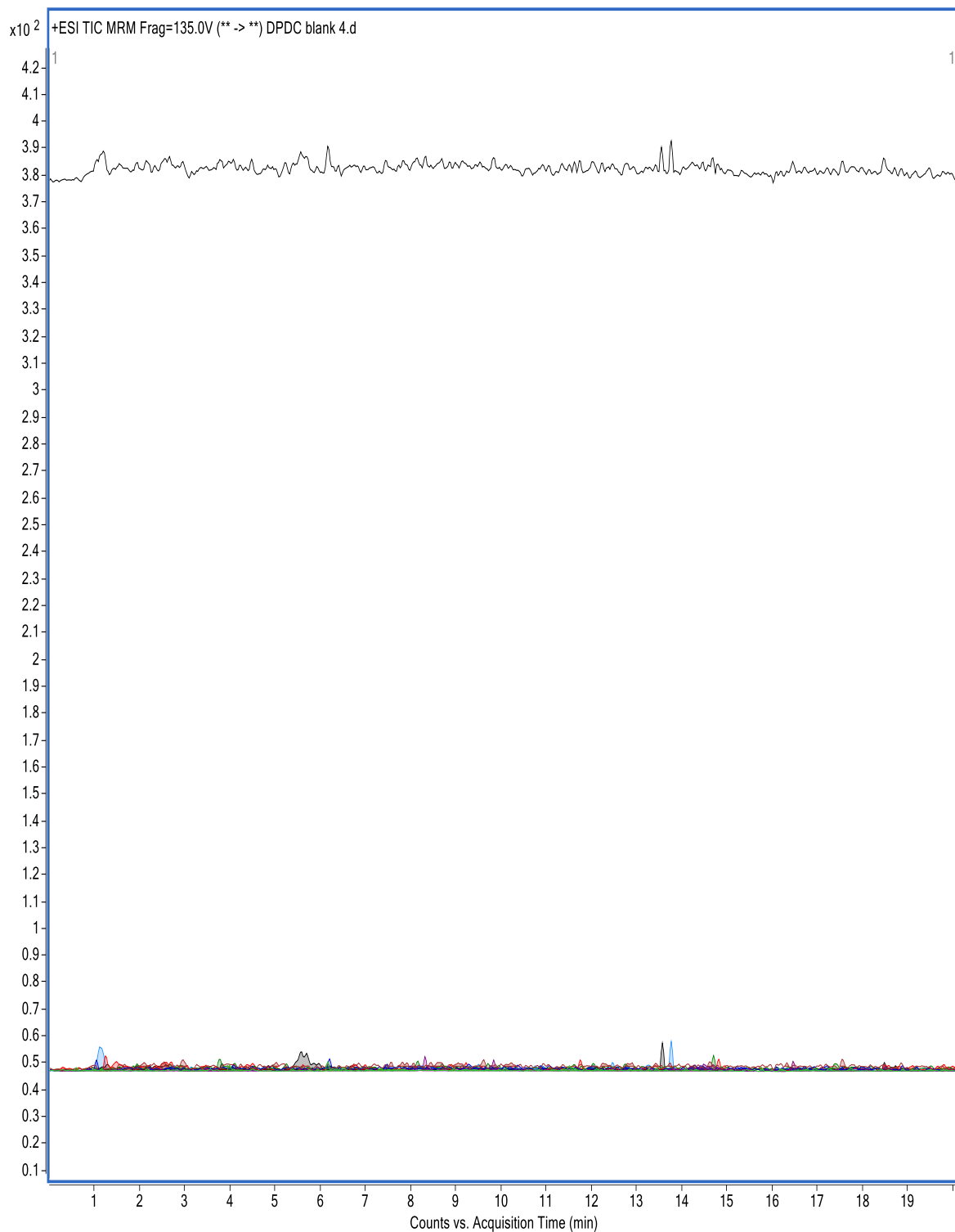


Figure 18: Chromatogram of recovery blank

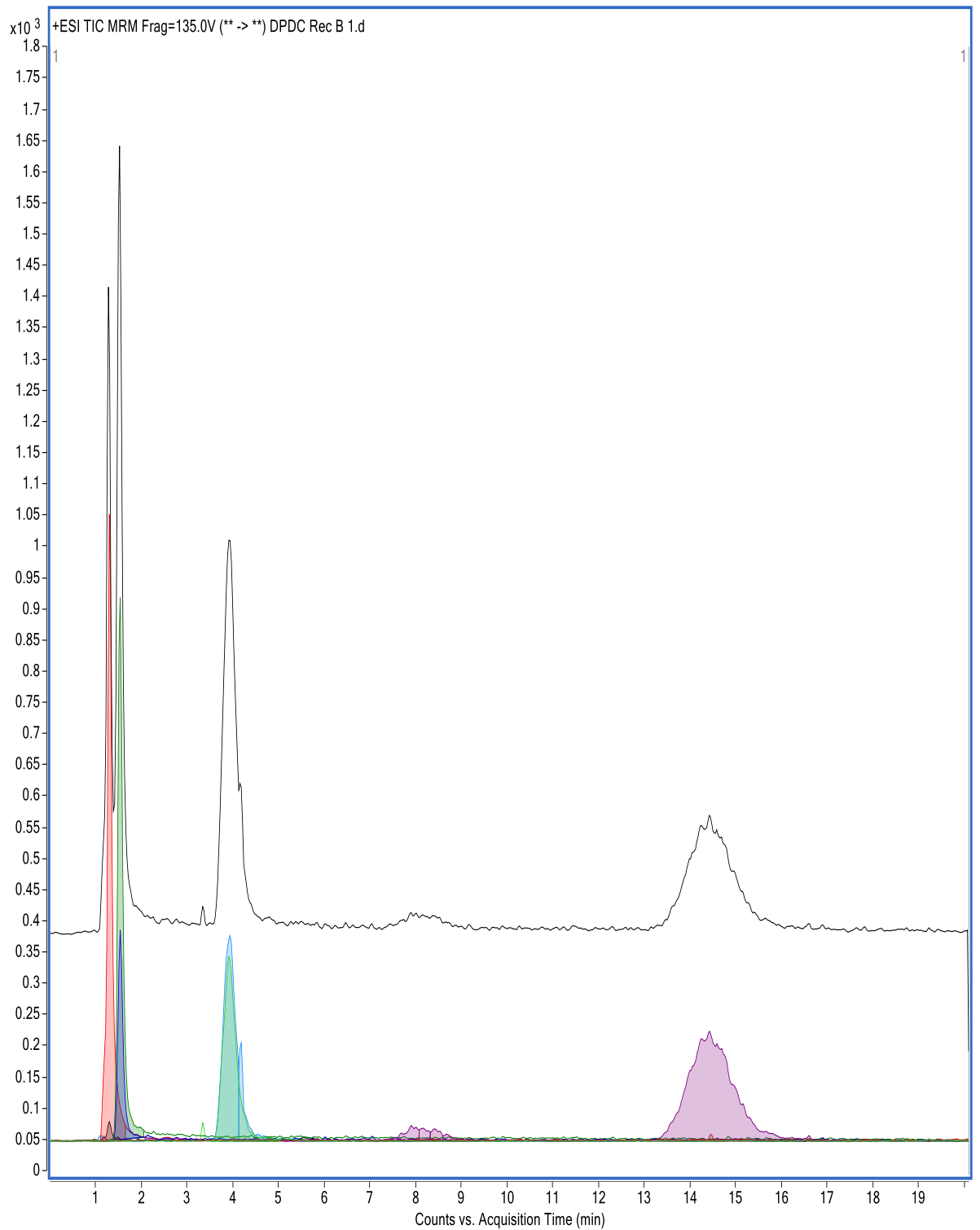


Figure 19: Chromatogram of recovery B (200 µg/kg spiked level)

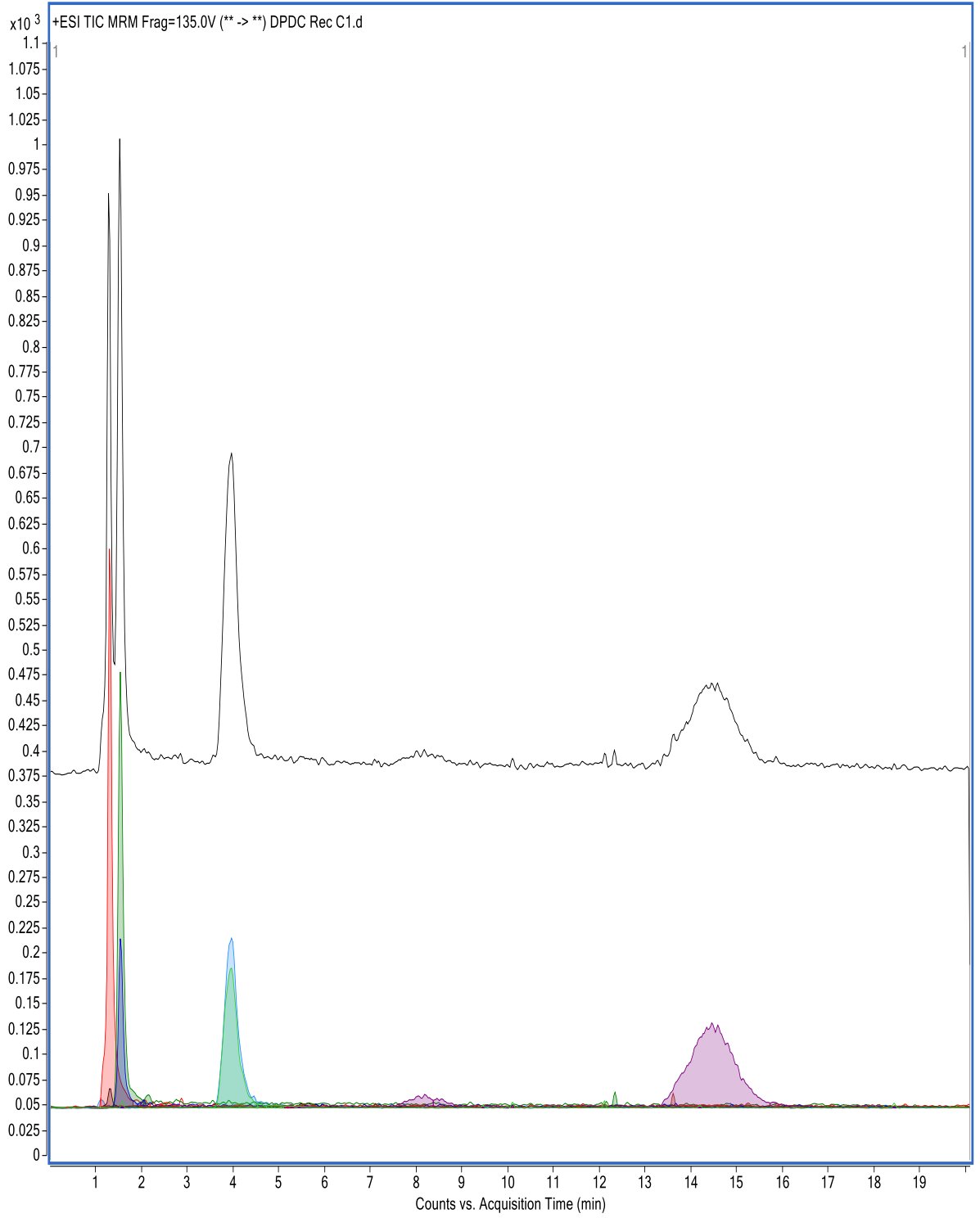


Figure 20: Chromatogram of recovery C (100 $\mu\text{g}/\text{kg}$ spiked level)

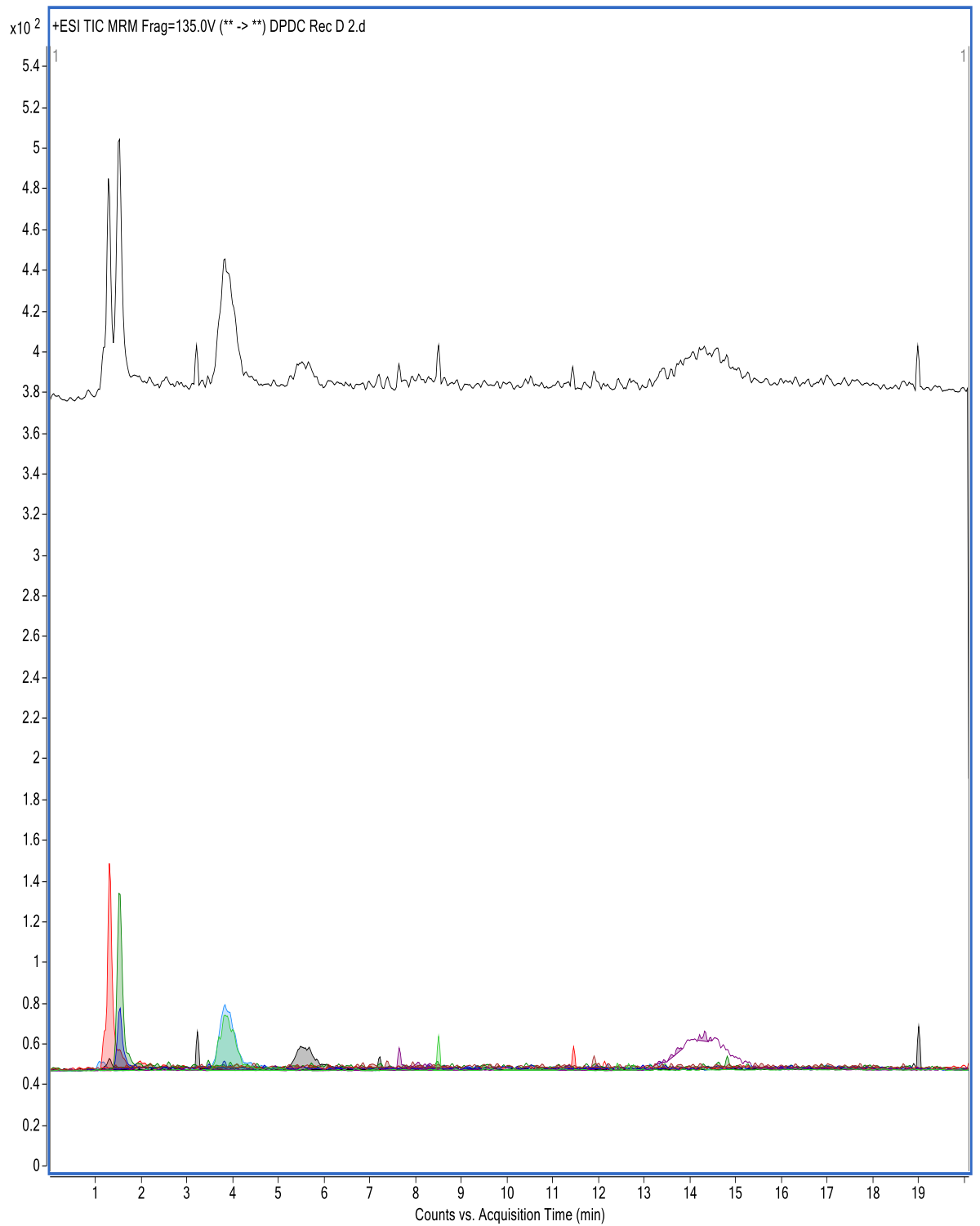


Figure 21: Chromatogram of recovery D (20 µg/kg spiked level)

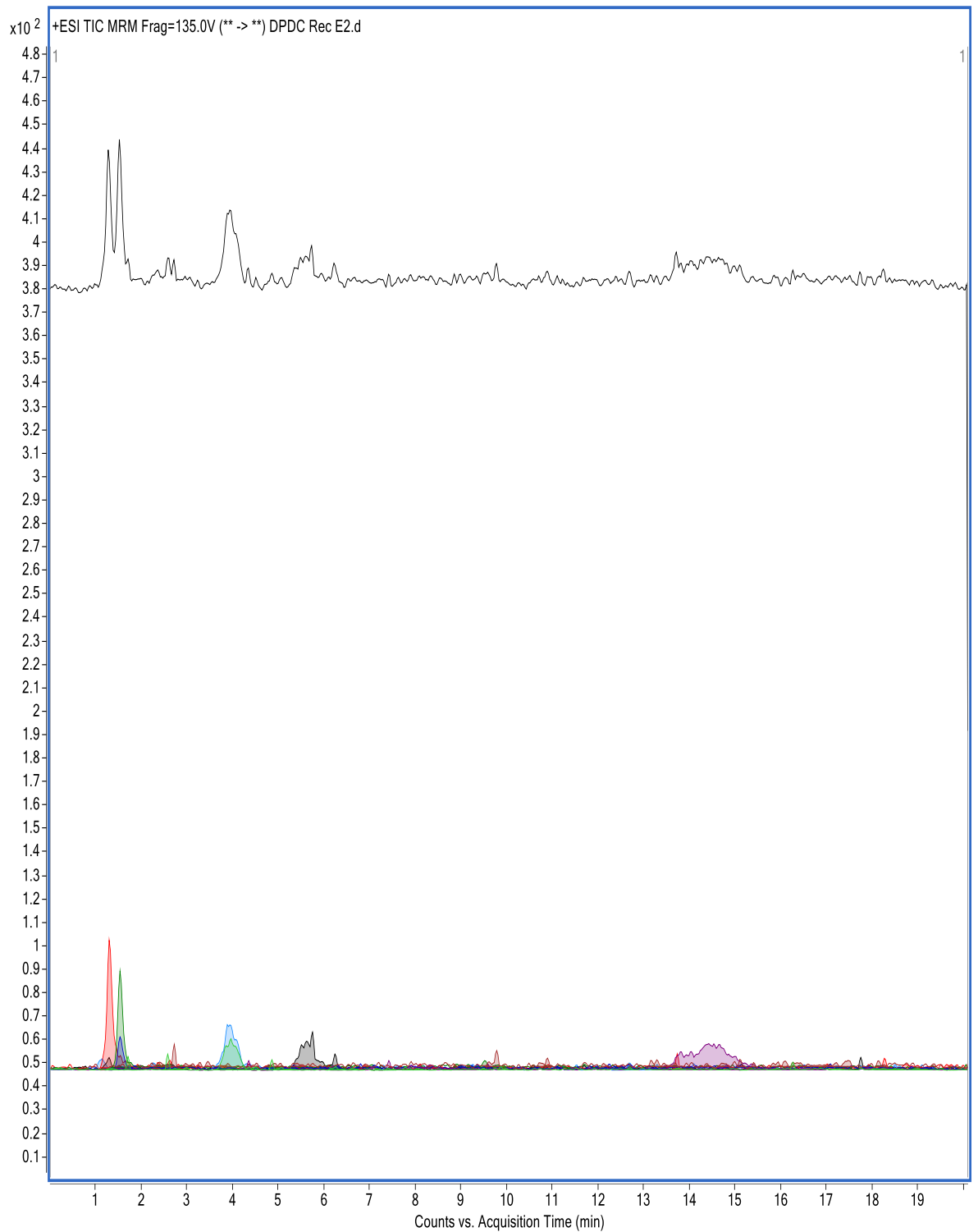


Figure 22: Chromatogram of recovery E (8 $\mu\text{g}/\text{kg}$ spiked level)



Figure 23: Brinjal collection