PURITY ANALYSIS OF IMIDACLOPRID INSECTICIDE COLLECTED FROM JESSORE DISTRICT BY ULTRA HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (UHPLC) WITH PHOTO DIODE ARRAY DETECTOR

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JUNE, 2022

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A Thesis
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 (MS)

IN

AGRICULTURAL CHEMISTRY

SEMESTER: JANUARY- JUNE, 2022

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CERTIFICATE

*This is to certify that the thesis entitled "**PURITY ANALYSIS OF IMIDACLOPRID INSECTICIDE COLLECTED FROM JESSORE DISTRICT BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (UHPLC) WITH PHOTO DIODE ARRAY DETECTOR**" submitted to the Department of Agricultural Chemistry, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE (M.S.)** in **AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bonafide research work carried out by KHONDOKAR MOHAMMED FIROZ AHMED, Registration No. 20-11151 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

JUNE, 2022

Dhaka, Bangladesh

(Dr. Md. Tazul Islam Chowdhury)
Professor
Supervisor

Dedicated to
My
Beloved Parents

ACKNOWLEDGEMENTS

The author seems it is a much privilege to express his enormous sense of gratitude to the Almighty Allah for there ever ending blessings for the successful completion of the research work.

The author wishes to express his gratitude and best regards to his respected Supervisor, Dr. Md. Tazul Islam Chowdhury, Professor, Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka, for his continuous direction, constructive criticism, encouragement and valuable suggestions in carrying out the research work and preparation of this thesis.

The author expresses his earnest respect, sincere appreciation and enormous indebtedness to her reverend Co-supervisor, Dr. Abdul Kaium, Associate Professor, Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka for his scholastic supervision, helpful commentary and unvarying inspiration throughout the research work and preparation of the thesis.

The author feels to express his heartfelt thanks again, to the honorable Chairman, Professor Dr. Mohammed Ariful Islam, Department of Agricultural Chemistry along with all other teachers and staff members of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka, for their co-operation during the period of the study.

The author wishes to extend special thanks to his class mates and friends for their keen help as well as heartiest co-operation and encouragement. The author feels proud to express his deepest and endless gratitude to Food Safety Laboratory of Sher-e-Bangla Agricultural University for the help of analysis of this research work.

The author expresses his heartfelt thanks to his beloved wife Dr. Nazma Pervin, parents, two beloved sons Yusha and Faeez and all other family members for their prayers, encouragement, constant inspiration and moral support higher study. May Almighty bless and protect them all.

The Author

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ABSTRACT

The present study introduces a UHPLC method that is simple, specific, and accurate in measuring the active component imidacloprid in the 20SL formulation. The analytical separation was conducted using a Waters Cortecs C18 column using an ultraviolet photodiode array (UV-PDA) detector at a wavelength of 272 nm. The method's validation results showed that the suggested method has good specificity with satisfactory linearity ($R^2 \geq 0.9994$), short retention time, and peak area precision. The method's accuracy and precision revealed a significant recovery (98.9 - 101.2%) at the three-spiked level with excellent precision (less than 1.89% RSD). The chromatographic separation took around 5 minutes to complete under the established chromatographic conditions. The developed and validated method was used to determine the purity of mostly used and available of seven (7) selected imidacloprid marketed brands insecticide from twenty one (21) samples. The samples were collected from sadar upazilla of Jessore district during September 2021 and then the samples were carried to the food safety laboratory of the Sher-e-Bangla Agricultural University, Dhaka on the same sampling day. Among 7 different brands of imidacloprid insecticide samples, 5 (71.43%) were found 110-130% pure which means over standard of active ingredient (AI). Whereas one of the total tested brand was 80-90% pure, and the remaining one brand was below 10% pure. This means that farmers are using imidacloprid having either higher or less of AI than required. This study reflects the small scale scenario of the presence of AI in different marketed brands of imidacloprid insecticide, which will help the policy maker to be aware of quality control importance of the plant protection products.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

ADI	Acceptable Daily Intake
ACH	Acetylcholine
APCI	Atmospheric Pressure Chemical Ionization
ASE	Accelerated Solvent Extraction
AOAC	Association of Official Analytical Chemists
BARI	Bangladesh Agricultural Research Institute
ChE	Cholinesterase
CSN	Committee for Standardization
DAS	Days After Spray
DLLME	Dispersive Liquid–Liquid Micro Extraction
DMCb	Dhaka Market Cabbage
DMBG	Dhaka Market Bitter Gourd
DV	Daily Value
d-SPE	dispersive Solid Phase Extraction
ECD	Electron Capture Detector
EPA	Environmental Protection Agency
ESI	Electrospray Ionization
et al.	*et alibi* (and others)
etc.	*et cetra* (and so on)
ECD	Electron capture Detector
EU	European Union
FAO	Food and Agriculture Organization
FID	Flame Ionization Detector
FPD	Flame Photometric Detector
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FTD	Flame Thermionized Detector
GAP	Good Agricultural Practices

GCB	Graphitized Carbon Black
GC-MS	Gas Chromatograph-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
HRI	Hazard Risk Index
KMRL	Korean Maximum Residue Limits
LC-MS	Liquid Chromatography-Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MCS	Multiple Chemical Sensitivity Syndrome
MDQ	Minimum Detectable Quantity
MRL	Maximum Residue Limit
MRM	Multiple Reaction Monitoring
NPD	Nitrogen-Phosphorus Detector
NPTN	National Pesticides Telecommunications Network
NTE	Neuropathy Target Esterase
PDA	Photodiode Array detection
PDI	Potential Daily Intake
PSA	Primary Secondary Amine
PHI	Pre-Harvest Interval
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RSM	Response Surface Methodology
RTL	Retention Time Locked
SAU	Sher-e-Bangla Agricultural University

CHAPTER I

INTRODUCTION

The agricultural industry in Bangladesh is a significant contributor to the country's economy, representing approximately 21% of the national gross domestic product (GDP)(Clarke et al., 2015). Agriculture is important to the economy of Bangladesh, as agriculture employs more than 80% of the population. Since agricultural land is decreasing, the main priority has been to increase crop productivity by adopting cost-effective technologies, using irrigation, chemical fertilizers, and pesticides. (Mukhopadhay, 2005). Farmers employ various pesticides to prevent crop damage caused by pest infestation. The application of pesticides in agricultural practices has experienced a significant global expansion since the 1950s. According to Yudelman et al. (1998), the Asia/Oceania region noticed an estimated annual compound growth rate of 4.4% from 1993 to 1998. Although pesticide use and crop loss due to pests are increasing globally, farmers tend to benefit from a small increase in pesticide use (Yudelman et al., 1998). The development of pest resistance to pesticides is a significant drawback associated with the usage of pesticides. This issue is further exacerbated by the general assertion that these chemicals adversely affect human health and the environment (Pingali, 1995; Aktar, 2017; Ami, 2014; Anonymous 2001). It is widely acknowledged that the rapid development of modern agricultural technologies has increased the use of pesticides (Roger and Bhuiyan, 1995; Pingali and Rola, 1995). Consequently, due to the widespread adoption of 'green revolution' technology in various regions of Asia, Latin America, and Africa, pesticides have emerged as a significant and enduring element of modern agriculture.

Bangladesh's usage of pesticides has increased dramatically since the 1970s when it was practically nonexistent. According to Rahman and Thapa's (1999) findings, there was a notable rise in pesticide usage from 2200 metric tonnes during 1980-1982 to 6500 metric tonnes during 1992-1994. Additionally, modern rice cultivation experienced a significant increase from 20.3% to 49.0% of the total rice area during the same period. Pesticides are now indispensable in improving agricultural output due to their rapid action, easy application, and availability (Chowdhury et al., 2011). In agriculture, the use of pesticides is an important

component for the effective management of field crops and stored grains, as it helps to mitigate the risks of potential losses resulting from insect pests and diseases. They are used to reduce the loss of crops due to vector-borne diseases and to increase crop yield and quality. Thus, pesticides and allied agrochemicals have become integral to sustainable agriculture (Kabir et al., 2008; BARC, BARI, 2001; Ballantyne and Marrs, 2017).

The use of pesticides in Bangladesh has shown an upward trend, with figures indicating a rise from 758 metric tonnes in 1960 and 3028 metric tonnes in 1980 to over 19000 metric tonnes in 2000, as reported by Hasanuzzoha (2004). According to Ali's (2004) findings, examining the growth rate of pesticide consumption over 24 years revealed an average annual increase of 9.0%. According to an anonymous source, in 2007, the sales of pesticides in Bangladesh amounted to more than 37,712.20 tonnes. It could also be attributed to impurity and adulteration of the pesticides used. According to Kabir et al. (2008), if the formulated pesticides contain fewer active ingredients, they may not be effective against insect pests, leading farmers to use more pesticides to achieve the expected results. Substantial data indicate the improper application of pesticides in Bangladesh. In a survey conducted across multiple districts in Bangladesh, 820 farmers were assessed, revealing that 70% of the pesticides employed were categorised as "extremely or very hazardous". Additionally, 47% of the farmers were identified as engaging in excessive usage of these pesticides. According to Prodhan et al. (2018), a mere 4% of farmers who participated in the survey disclosed that they had received minimal instruction on properly handling pesticides. Furthermore, 87% of these farmers acknowledged not employing any protective measures while mixing and handling pesticides (Prodhan, 2018a; Prodhan, 2018b; Prodhan, 2010; Prodhan, 2009; Marina, 2020 & 2019).

Nonetheless, adulterated pesticides may fail to achieve their intended purpose while posing a potential threat of phytotoxicity to the treated crops and posing a risk to human health and the environment. The demand for high-quality pesticide products is significant. In this context, specifications play a critical role in enhancing the quality of pesticides. Impurities in pesticides typically arise from precursor compounds or unintended reactions during the manufacturing process and during the storage of the final product. The indiscriminate and disproportionate

application of pesticides has resulted in many ecological and societal concerns, including the degradation of agricultural ecosystems and the emergence of resistance in insect pests, pathogens, and weeds (Begum et al., 2016; Chowdhury et al., 2013; Dhas and Srivastava, 2010). It has been claimed that the regulatory process for pesticide registration is systematic, based on information from the national report originally compiled by FAO (2011) Corporate Document Repository. In practical terms, there exists a discrepancy between policies and their execution. Although the intention behind the ordinance and regulations to monitor formulations and residue is praiseworthy, inadequate facilities and a shortage of trained analysts hinder proper monitoring. Thus, the specifications of pesticides on the market may differ from those registered (Aziz, 2005 & 2006).

Thus, it is hypothesised that pesticide adulteration is a primary reason for the widespread application of pesticides in Bangladesh. Substandard or insufficient active ingredient (AI) designed pesticides do not perform against insect pests and diseases, and farmers must use more pesticides to achieve the desired results. Impurity and adulteration may be one of the causes of excessive and repetitive pesticide usage in crops, as well as a loss in insecticide efficacy (Anonymous, 2009 & 2019). This study was undertaken with the following objectives:

1. To develop and validate the UPLC-PDA detection method for purity analysis of Imidacloprid insecticide.
2. To assess the active ingredient present in the imidacloprid insecticide collected from Jessore district.

CHAPTER II
REVIEW OF LITERATURE

It is assumed that impurity or adulteration of pesticide might be one of the major causes of extensive use of pesticide in Bangladesh. Impure pesticides may less or no effective for the control of insect pest and diseases. In this perspective, it has become important to analyze the marketed brands of insecticides for the determination of percent purity and to find out the actual amount of active ingredient remain in the marketed brands. With this respect some review of literature is presented below:

2.1 Toxicity of pesticides

Organophosphate pesticides have been shown to adversely affect the photo-synthesis (Zobiole *et al*., 2012; Kielak *et al*., 2011) plant mineral nutrition (Zobiole *et al*., 2010), carbon metabolism (Ding *et al*., 2011; Zobiole *et al*., 2011), photochemical reactions (Vivancos *et al*., 2011), chlorophyl biosynthesis (Serra *et al*., 2013), fatty acids synthesis, amino acids synthesis, nitrogen metabolism (Zobiole *et al*., 2010), and oxidative stress (Vagi *et al*., 2018).

Organophosphorus prevents the biosynthesis of catalase, perioxidase and 5-aminolevulinic acids (ALA) which are the major component of chlorophyl biosynthetic pathway by inducing Fe deficiency in plants (Barcelos *et al*., 2012). However, it affects ALA production by competing with the major product of the ALA synthetase active site or leading to deprivation of glumate content by competing with glycine in the photorespiration process as depicted in soybean (Vivancos *et al*., 2011). They also reduce the availability of amino acids and metal ions which are associated with PSI and PSII to transfer photon (light energy) into the electron transport chain system (Cakmak *et al*., 2009).

Foliar spray of glyphosate and its metabolites decreases the net stomatal conductance and carbon exchange in plants thus reducing the CO_2 assimilation capacity (Zobiole *et al*., 2011; Ding *et al*., 2011). Exposure of organophosphate pesticide, glyphosate also lower down the levels of 3- phosphoglyceric acid (PGA) and ribulose-1,5-biphosphate (RuBP) affecting ribulose 1,5-biphosphate carboxylase oxygenase activity (Rubisco) in plants (De-Maria *et al*., 2006).

The reduction of plant growth due to OPs could also be possibly due to effect on cell division and elongation. Mishra *et al*. (2015) reported delayed seedling emergence and reduced lumber of *Vigna radiata* upon treatment with phorate. Various concentrations of chlorpyriphos in the range from 0 to 1.5 mM were spiked on twenty-day old plants of V. radiata through foliar spray. Analyses was done at pre-flowering stage (Day 5), flowering stage (Day 10) and post flowering stage (day 20) after the treatment. Chloropyrifos was found to reduce plant growth and nitrogen metabolism in *V. radiata.*

Excessive use of organophosphate pesticides affects the non-target crops and non-target animal species found in various aquatic and terrestrial ecosystems (Blann *et al*., 2009).

The interactions of the pesticides with soil organic inorganic component at the molecular level are central to their bioavailability, bioaccumulation, transport and toxicity in the environment (Morton and Edwards, 2005).

Profound understanding of the soil's interactions with pesticides is crucial to understand the soil-pesticide-minerals, soil-pesticide- organic matter, soil-pesticide-plant and soil fertility mechanisms (Polubesova and Chefetz, 2014). OPs are known to interact on soil's mineral surface and organic matter present in soil (Dror *et al*., 2017; Scheunert, 2018).

The interaction of pesticides to soil depend upon four main factors; nature of solute (pesticide), solvent (mainly water), soil constituents and pH (Gianfreda and Rao, 2008). In general, mineral surface is positively charged at low pH and

negatively charged at high pH values. As a result, positively charged metal ions interact to mineral surface at moderate acidic to basic pH. The negative charged ligands interact to soil's minerals surfaces at low pH only.

The high rate of pesticide usage in Bangladesh may be attributed to the presence of impurities or contaminants in the pesticides. Impure pesticides could show reduced or negligible efficacy in managing insect pests and diseases. From this standpoint, it has become imperative to analyze the insecticide brands available in the market to ascertain their percentage purity and determine the precise quantity of active ingredients present in them. In this regard, a literature review is provided below:

2.2 Imidacloprid pesticide

In the last 30 years, neonicotinoids that block nicotinic acetylcholine receptors have been the most quickly developed pesticides in modern crop protection. They are used against a wide range of sucking and chewing pests (Handa, 1996; Hardin 2018; Hassan et al., 2017; Hossain et al., 2014). Imidacloprid a neonicotinoid pesticide has attained generic status in most Afro-Asian nations and has emerged as the most efficacious and top-selling insecticide globally. Neonicotinoids have gained popularity as a replacement for various chemical groups of insecticides, such as pyrethroids, chlorinated hydrocarbons, organophosphates (OPs), and carbamates, which were previously used to manage insect pests on major crops. This is due to their effective and noble mode of action on the insect central nervous system (CNS) (Nahar, 2020; Price, 2008; Polubesova, 2014).

Imidacloprid is a neurotoxic insecticide that exhibits a high affinity for nicotinic acetylcholine receptors (nAChRs) and impedes the activity of acetylcholine, a naturally occurring neurotransmitter, thereby disrupting normal nerve function (Kabir, 2007; Kabir et al.,1996; Kabir, 2008a). Imidacloprid has garnered significant scientific and public interest due to its long tenure on the market than other neonicotinoids. Furthermore, Bayer Crop Science's patent on imidacloprid expired in 2006, allowing other manufacturers to develop a generic version of the insecticide at a substantially reduced cost. As a result of its low cost and long-term efficacy, imidacloprid is the most extensively used pesticide in agriculture and forestry.

2.3 Methods of Detection of Pesticides

The development of various analytical techniques has allowed us to deduce information and determine the structure of a newly synthesised molecule.

Currently, there is ongoing research aimed at identifying appropriate detection and quantification techniques for detecting pesticides using various spectroscopic techniques such as UV-Vis (Yuan et al., 2015), FTIR (Du et al., 2010), mass spectrometry (Lorenzo and Pico, 2017), X-ray diffraction (Gong et al., 2012), electrochemical, and NMR spectroscopy (Hiscock et al., 2015; Kumar et al., 2013).

Pesticide detection and estimation in water (Borjesson and Torstensson, 2000), guava fruit extract, soil (Peruzzo *et al*., 2008), sediments (Aparicio *et al*., 2013), animal and human tissues (Kruger *et al*., 2014), plants tissues (Nedelkoska and Low, 2004), wheat grains (Jan *et al*., 2009), urine samples, organs of dairy cow (Kruger *et al*., 2014), soybean extracts (Arregui *et al*., 2004) and carrot (Kataoka *et al*., 1996).

2.4 High performance liquid chromatography (HPLC)

Initially, HPLC techniques were devised to detect pesticide residues in non-volatile or thermally unstable substances. HPLC methods' increasing acceptance and applications can be attributed to their ability to provide a simpler and faster approach for analysing a wide range of compounds, as reported in the literature (Martinez et al., 1992). According to the literature, HPLC techniques are typically preferred over GC techniques due to their suitability for polar and thermally labile compounds (Lesueur et al., 2008). The process commonly involves diverse stationary phases, a pump that facilitates the movement of the mobile phase(s) and analyte through the column, and a detector that provides a specific retention time for the analyte. The retention time of an analyte is subject to variation based on the intensity of its interactions with the stationary phase, the ratio and composition of the solvent(s) used, and the flow rate of the mobile phase. High-performance liquid chromatography (HPLC) uses a pump instead of gravity to generate the elevated pressure necessary for propelling the mobile phase and analyte through the densely packed column. The heightened density is a result of reduced particle dimensions. In practical terms, this technique

enables more effective separation of columns with shorter lengths than conventional column chromatography (Jin et al., 2012).

Multiple detection methods, such as UV absorption, UV photodiode array (PDA), fluorescence (FL), and chemiluminescence (CL), are used in HPLC techniques to determine pesticide residues (Wu et al., 2002). The use of liquid chromatography (LC) in conjunction with a photodiode array detector (PDA) presents a non-invasive approach that affords numerous benefits. In addition to its capacity for multi-wavelength monitoring, this detector facilitates the comparison of the ultraviolet spectrum of a targeted peak with a repository of ultraviolet spectra, thereby aiding in the identification and confirmation of the stated peak. A challenge associated with UV detection pertains to pesticides exhibiting significant absorption at wavelengths that fall below 250 nm, which is also the spectral region where numerous reactive and matrix-derived interferences absorb. LC-UV analysis is frequently used in formulations with high concentrations or extremely clean substrates, as stated in reference (Sanz et al., 2004).

2.4 Purity analysis of pesticides

Imidacloprid is a neonicotinoid pesticide used in crop production to control termites, aphids, bugs, and grubs (Sheets, 2003). Multiple application formulations of Imidacloprid have been reported in the literature, including foliar sprays, seed dressings, and soil treatments (Zhao et al., 2020). The present study provides an overview of the purity analysis of imidacloprid insecticide obtained from the Jessore district. The study includes a review of relevant literature on the subject:

Begum et al. (2016) investigated the purity of nine different pesticides, including chlorpyrifos, diazinon, carbofuran, pyrazosulfuranethyle, dimethoate, cypermethrin, carbendazim, mencozeb, and quinalphos. The present study involved the collection of pesticides from local markets in eight distinct locations in Bangladesh, namely Rajshahi, Rangpur, Dinajpur, Bogra, Chittagong, Mymensing, Comilla, Norshingdi, and Jessore districts. These locations were selected due to the high prevalence of pesticide usage in the region. Out of the 66 pesticides that were subjected to testing, 44 of them, which accounts for 66.66% of the total, were discovered to possess a purity level of 90% in relation to their active ingredient (AI). Approximately 12% of

the brands that underwent testing exhibited a purity range of 80% to 90%, while the remaining 21.34% demonstrated a purity level below 80%. Notably, three of the pesticide brands tested were found to contain no active ingredient whatsoever.

Ahmed et al. (2016) conducted a study to assess the purity of various commercially available brands of three insecticide groups: quinalphos, Malathion, and fenitrothion. The study was conducted at the Pesticide Analytical Laboratory, w part of the Division of Entomology at the Bangladesh Agricultural Research Institute (BARI) in Gazipur. The researchers utilised appropriate protocols involving GC-FID for their analysis. This study analysed and estimated the purity of nineteen insecticide brands obtained from dealers or retailers in the Jessore, Gazipur, and Rangpur regions over two seasons, namely 2006-2007 and 2007-2008. During the two seasons under study, it was observed that out of the five commercially available brands of Malathion, only one brand (MTF) exhibited a purity range of 98.95-100%. This plant was sourced from Jessore in 2007-08 and Gazipur in both seasons and was deemed a standard or acceptable product. However, in other regions, this brand wacontainedess an active ingredient (AI) and exhibited a purity level of less than 95%, which was classified as a substandard product. The residual brands exhibited purity levels ranging from 22% to 92%, with SRL and MTX containing minimal AI (22-44% purity). These findings indicate that all brands above were deemed impure and unsatisfactory. The present study observed the efficacy of fenitrothion, which is available in five distinct commercial brands. In 2006-07, the SMT brand achieved a purity level of 96%, exclusively in Gazipur and Jessore. The following year, the brand maintained this high purity level across all three locations, thereby establishing itself as a standard product. In all locations and throughout two seasons, the remaining four brands of this particular insecticide exhibited substandard levels of purity. In all locations during the 2006-07 and 2007-08 seasons, only MLX and BLX were the marketed brands of Quinalphos, with eight brands available. The product was of standard quality with a purity level of 95%. In t006-07, ALX and CRX exhibited a high degree of similarity to MLX, except for a single location, namely CRX in Gazipur and ALX in Jessore. However, during the subsequent season, both brands were found to be substandard and impure, with purity levels ranging from 65-86% across all locations. The other brands, namely KNX, QNP, VNR, and SLX,

exhibited substandard and impure characteristics, with purity levels ranging from 59% to 87%.

The study conducted by Ahmed et al. (2017) aimed to assess the level of purity of various commercially available brands of three insecticides, namely diazinon, acephate, and cypermethrin, through the utilisation of appropriate protocols such as GC-FID and GC-ECD. The present study involved the analysis of nineteen commercially available insecticide brands obtained from retailers located in the Jessore, Gazipur, and Rangpur regions. The objective was to estimate the purity of these insecticides during two distinct seasons, namely 2006-2007 and 2007-2008. Out of the six diazinon brands available in the market, only the RSN brand exhibited a purity level ranging from 96.71% to 100% in both seasons and across all locations, thereby meeting the standard product criteria. However, in other regions, four brands (DZN, SBN, HZN, DNN) were found to contain less than 95% pure, indicating that they were substandard products. In the years 2006-07, the brands DNN and AZN exhibited a purity range of 33.71-51.94%, while the brands SBN and DZN demonstrated a minimal quantity of active ingredient (0.16-0.84% purity) in 2007-08. All of these brands were deemed to be of inferior quality due to their impurities. In the year 2006-07, it was observed that all five brands of acephate that were tested had a purity range of 57.14-88.59%, indicating that they were below standard. However, in the following year (2007-08), three brands (SNT, BNS, ATF) demonstrated a purity level exceeding 90%, while the remaining two brands had a purity level below 80%, indicating that they contained less active ingredient than required. In the year 2006-07, a total of eight brands of cypermethrin were subjected to testing, out of which three brands were found to have a purity level of >95% across all locations. The two additional brands, namely CPR and AMT, exhibited a purity level exceeding 90%. During the period of 2007-08, it was observed that two specific brands of cypermethrin, namely RCD and SCR, exhibited a purity level exceeding 95%. On the other hand, two other brands, CRN and RLT, demonstrated a purity range of 88.77-91.15%. In 2006-07, the UTD brand was deemed to be of standard purity. However, in the subsequent year of 2007-08, this brand was found to be substandard in quality across all locations. The majority of the cypermethrin brands that were tested

exhibited varying levels of purity, with some being deemed substandard, when compared to diazinon and acephate.

A study was conducted by Islam et al. (2016) to assess the purity of nine distinct available on-market brands of carbofuran. These brands were collected from various locations in Gazipur, Bangladesh. The purity of the samples was determined using the HPLC 20A Prominence method. Carbofuran is a systematic insecticide utilised to manage various field crops. Its chemical name is 2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate. A standard calibration curve was generated by plotting the area of a standard carbofuran solution against varying concentrations (ppm) using the GCMS QP-2010 instrument manufactured by Shimadzu Corporation in Japan. Of the nine brands analysed, four exhibited complete purity, three demonstrated purity levels exceeding 80%, and two exhibited purity levels below 70%, about the established standard for carbofuran. The findings of this research would benefit dealers, consumers, and farmers in gaining knowledge regarding the varying levels of carbofuran content present in different brands within the Bangladeshi market.

Marina et al. (2019) conducted a study to determine the actual scenario of eleven selected pesticides collected from local marketplaces in seven distinct areas around Bangladesh. The present investigation employed Gas Chromatography in conjunction with Flame Ionisation Detector (FID) and Electron Capture Detector (ECD) to ascertain the purity of acephate, diazinon, dimethoate, chlorpyrifos, quinalphos, malathion, cypermethrin, fenvalerate, and fenitrothion. Additionally, High-Performance Liquid Chromatography (HPLC 20A Prominence) coupled with Photo Diode Array (PDA) detector was utilised to determine the purity of carbofuran and carbosulfan. Ninety-five distinct pesticide brands were gathered and subjected to analysis. Out of the brands that were subjected to testing, it was found that 57 of them contained 100% AI. On the other hand, 31 brands tested for various pesticides contained between 80-99% AI, while seven brands tested for different pesticides contained less than 80% AI.

CHAPTER III
MATERIALS AND METHODS

Samples of imidacloprid insecticide from various marketed brands were collected from sadar upazilla of the Jessore district. Total 21 samples were collected from seven brands during September 2021. Then the collected samples were sent to the food safety laboratory of Sher-e-Bangla Agricultural University in Dhaka, Bangladesh, for purity testing. The following procedures cover all necessary steps from sampling to final analysis.

3.1 Chemicals and reagents

Standard imidacloprid (99.5% pure) and LC-MS grade acetonitrile (ACN) were obtained from Sigma Aldirch, Germany, through Kuri and Kuri Company, Dhaka, Bangladesh. In this study, different marketed brands of imidacloprid insecticide were collected from different market of Jessore City. The solvents underwent filtration using a Solvent Filtration Apparatus 58061 from Supelco (Bellefonte, PA, USA) equipped with 0.22 μm membrane filters. The UHPLC mobile phase and other aqueous solutions were prepared using ultrapure water obtained from a Thermo water purification system. Before analysis, the final sample was filtrated using 13 mm filters equipped with a PTFE membrane of 0.22 μm pore size.

3.2. Instruments and Equipments

The Shimadzu Nexera LC-40D XS Ultra High-Performance Liquid Chromatography (UHPLC) system, manufactured by Shimadzu Corporation in Japan, was used for the chromatographic analysis. The system was fitted with a vacuum degasser (DGU-403), a binary pump (LC-40D-XS), an autosampler (SIL-40C XS), a thermostatted column compartment (CTO-40C), and a UV-VIS Photodiode array detector (SPD-M40). The process of acquiring data was executed using the LabSol LC Connect (223-10100-54) system. The analytical column used in the investigations was the Waters Cortecs C18 (150 mm x 4.6 mm, 2.7 μm, Waters Corporation, Milford, USA). The Shimadzu analytical balance (model ATY324, Shimadzu Corporation, Japan) was used to measure the weight of the standard to prepare the standard stock solution of imidacloprid. To enhance the dissolution of the stock solutions, we used an ultrasonic

bath, specifically the "Elma" model, and a vortex mixer, namely the "Digisystem VM 2000". In addition to the above instruments the following accessories were also used:

(a) Measuring cylinder
(b) Conical flask
(c) Volumetric flask
(d) Funnel
(e) Test tube
(f) Micro pipette
(g) Aluminum foil
(h) Para film
(i) Glass vial
(j) 0.22 µm filter paper etc.
(k) Scissor

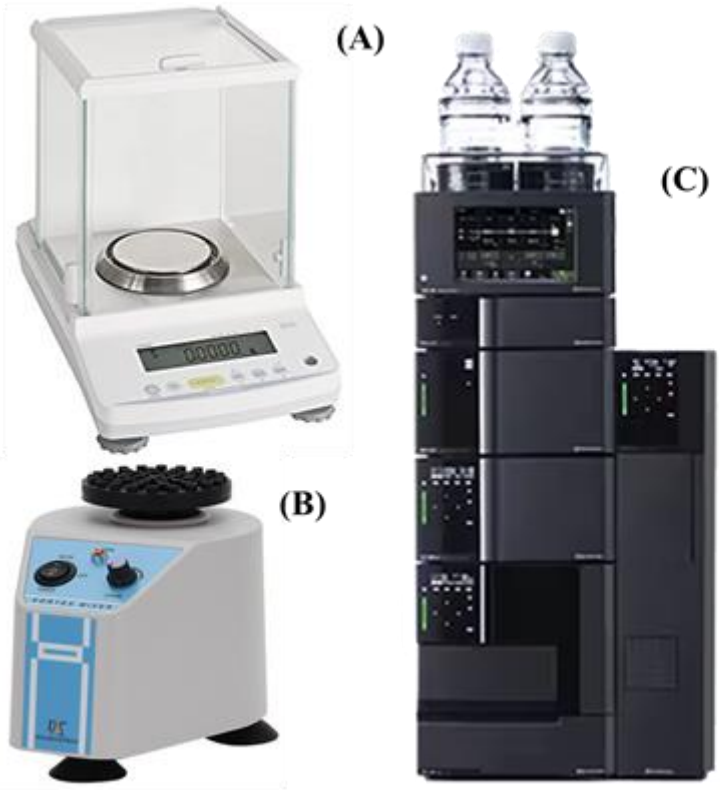

Figure 1. Instruments (A) Analytical balance, (B) Vortex mixer and (C) Shimadzu UHPLC used for the sample preparationa and analysis of imidacloprid insecticide.

3.3. Preparation of Standard Solutions

A solution of imidacloprid stock (1000 mg/L) was prepared using acetonitrile (ACN) of LC-MS grade. The standard working solutions (5, 10, 15, 20, and 25 mg/L) were produced from standard stock solutions in LC-MS grade ACN. The solutions were kept at a temperature range of 4-7 ºC and kept away from exposure to light.

3.4. Imidacloprid 20 SL

English name: Imidacloprid
Chemical name: 1-((6-chloro-3-pyridinyl)methyl)-N-nitro-2-imidazolidinimine
Chemical Structure: (Figure 1)
Chemical Formula: C9H10ClN5O2
Molecular weight: 255.66

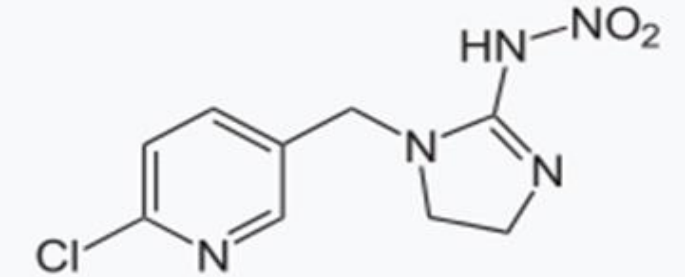

Figure 2. Chemical structure of imidacloprid insecticide.

Physical and Chemical properties:

The pure product is a white or colorless crystal with a weak odor, a melting point of 143.8 ℃ (crystal form 1), 136.4 ℃ (crystal form 2). At 20 ℃, the relative density is 1.543. Solubility: 0.51 g / L in water, 50-100 g / L in dichloromethane, 1-2 g / L in isopropanol, 0.5-1 g / L in toluene, less than 0.1 g / L in n-hexane, stable at pH 5-11. The content of active ingredients in the original drug is more than 80%, the appearance is light orange crystal, the melting point is 128-132 ℃, and the pH value is 6.5-7.5.

The insecticide Imidacloprid 20 SL is a neonicotinoid that is soluble in water. It contains 200g of active ingredient per 1L of formulated insecticide. It works by interfering with impulse transmission in insect nervous systems. The mechanism of

action involves the stimulation of specific neural cells through the activation of a receptor protein. This substance functions as both an acute contact and stomach poison. It is distinguished by its exceptional systemic characteristics.

3.5. Study area

The study area is Sadar Upazilla of Jessore district, which is the major vegetables producing hub of the southern region of Bangladesh. In this study, different marketed brands of imidacloprid insecticide were collected from different market of Jessore Sadar Upazilla.

3.6. Sample collection

The study involved the collection of a total of twenty-one (21) samples of 7 brands imidacloprid 20 SL insecticide. The sample was collected and placed in a separate ziplock box, which was then labelled with a distinct purpose of identification. Several commercially available brands of imidacloprid 20 SL insecticides were acquired. Table 1 displays the sample identification code and corresponding collection area for the selected commercially available imidacloprid brands.

Table 1. Sample Identification code along with the area of collection of the selected marketed brands of imidacloprid insecticide.

Name of the pesticides	Sample ID	Area of collection
Imidacloprid 20 SL	IDC-F1-R1 IDC-F1-R2 IDC-F1-R3	Jessore Sadar
	IDC-F2-R1 IDC-F2-R2 IDC-F2-R3	
	IDC-F3-R1 IDC-F3-R2 IDC-F3-R3	
	IDC-F4-R1 IDC-F4-R2 IDC-F4-R3	
	IDC-F5-R1 IDC-F5-R2 IDC-F5-R3	
	IDC-F6-R1 IDC-F6-R2 IDC-F6-R3	
	IDC-F7-R1 IDC-F7-R2 IDC-F7-R3	

*** IDC= Imidacloprid, F1, F2……F7= Different shops, R1, R2, R3= Replication**

3.7. Sample preparation

Prior to analysis, the sample was subjected to mechanical homogenization and subsequently stored at room temperature without any additional additives. The imidacloprid 20 SL label indicates that the formulation contains 200g of active ingredient per litre. In a volumetric flask with a 100 mL capacity, 10 mL of the imidacloprid 20SL formulated sample was taken; the contents were dissolved in 50

mL of acetonitrile, and the final volume was brought up to the mark with acetonitrile (final concentration 20 ppm). All samples were filtered through 0.22 µm PTFE Iso-Disk filter before HPLC analysis. The following parameters were used to inject the sample solutions onto the UHPLC:

3.8. UHPLC Parameters

High-performance liquid chromatography (HPLC) is commonly used to analyse pesticide-active compounds. Ultra-High-Performance Liquid Chromatography (UHPLC) is a technique used to separate distinct components of a substance, just like High-Performance Liquid Chromatography (HPLC).

Instrument	:	UHPLC (Shimadzu, Nexara LC-40D XS) with PDA detector
Pump	:	Binary pump with maximum operating pressure 105 Mpa
Column	:	C-18 (CORTECS, Waters), [150 mm x 4.6 mm, 2.7 µm]
Column Oven Temp	:	30ºC
Detector	:	Photo diode array (PDA) detector
Light Source	:	D2 Lamp
Injection Volume	:	10 µL
Software	:	LabSol LC-PDA

Imidacloprid

Wave Length	:	272 nm
Mobile Phase	:	Acetonitrile: Water (70:30, v/v)
Mode of Mobile Phase	:	Isocratic
Flow Rate	:	0.50 mL/min
Analysis time	:	5.0 min

UHPLC and UV-PDA were used to create a new analytical method for determining the amount of imidacloprid present in the 20SL pesticide formulation. Analyses were conducted using a Waters Cortecs C18 analytical column with dimensions of 150 mm × 4.6 mm and a stationary phase particle size of 2.7 µm. The choice of Cortecs C18 as the stationary phase was based on its remarkable efficiency, which allows for enhanced speed and resolution of the chromatographic process within the pressure range of current instruments. Furthermore, the improved resolution and speed of

modern UHPLC and LC-MS systems allow for operation at higher pressures, further enhancing the stationary phase's performance. The mechanical stability of these columns enables them to be used under conditions of up to 105 MPa pressure and a maximum temperature of 90 °C. The Waters Cortecs C18 is designed for reversed-phase chromatography and is suitable for analysing alkaline, neutral, or acid samples within a pH range of 2 to 9.

3.9. Method validation

3.9.1. Specificity and selectivity

The determination of specificity was based on the retention time of a specific pesticide standard on the chromatogram, which was subsequently compared to the retention time of the samples analyzed.

3.9.2. Linearity

Calibration curves were constructed by graphing the average peak area against the corresponding concentration of each substance. The linearity of the data was assessed through the computation of the correlation coefficient (R2), intercept, and slope of the regression line at five distinct concentration levels. The calibration solutions were prepared through the process of dilution of the respective stock solutions for each substance.

3.9.3. Accuracy and Precision

We calculated the accuracy or trueness of the method by reference to the label contents because no blank formulation was found. The mean recovery was calculated using five replicates. In the instance of repeatability, we calculated the precision (relative standard deviation repeatability (RSDr)) by analyzing five replicate samples on the same day.

3.10. Data analysis

The Shimadzu LC Lab Sol software automatically calculates the mg/L values for method optimization and purity analysis of the collected samples. The data analyses and calculations were performed using the MS Excel 2016 software.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Optimization of the analytical method

The performance of a chromatographic system can be improved by conducting a system suitability test, an essential technique in liquid chromatography aimed at enhancing resolution. Before developing our method, we conducted a system suitability assessment using a standard solution comprising imidacloprid standards. The system suitability parameters, such as absorption capacity, specificity, and selectivity, were evaluated at specific retention times.

The UV spectra of imidacloprid recorded in an acetonitrile and water solution (70:30, v/v) were used to determine the wavelength for the chromatographic studies. Imidacloprid's ultraviolet (UV) contour spectrum is shown in Figure 1b. The UV detection wavelength was set to the maximum absorbance range of 190-400 nm for the target compounds. The maximum absorption was recorded at 272 nm in the UV spectra of imidacloprid. Therefore, the chromatographic analysis for determining imidacloprid in the pesticide formulation was performed at a wavelength of 272 nm.

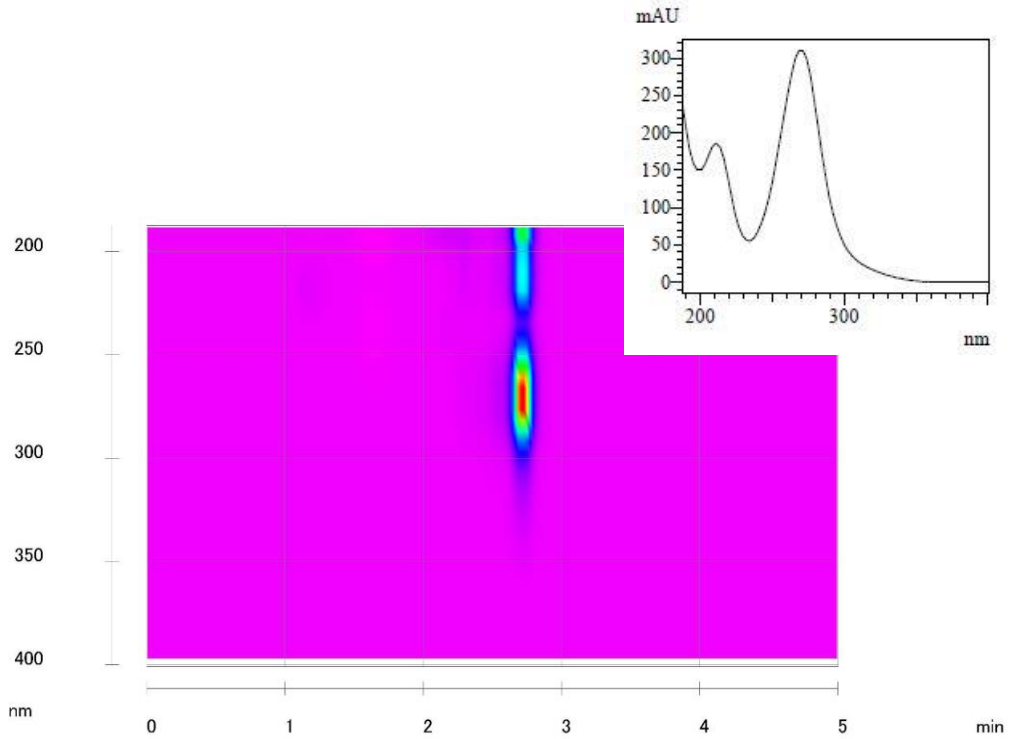

Figure 3. UV contour spectra and UV index of of imidacloprid standard of shows maximum absorption at 272 nm.

In order to develop a simple LC method for determining the active ingredient of imidacloprid in a pesticide formulation, the chromatographic procedure was carried out by isocratic elution, which involves using a mobile phase with a consistent composition. A set of experiments was conducted to determine the optimal conditions for chromatographic analysis of imidacloprid. In this experiment, we conducted the mobile phase optimization test on the ratio of 50/50%, 60/40%, 70/30%. 80/20% and 90/10 % of ACN/water. During the experiments, the peak area of 20mg/L imidacloprid standard was varied with different ratio of ACN/Water as mobile phase in chromatographic separation. The mobile phase solvent for separating the imidacloprid active ingredient was optimised and selected based on its greater peak area, with a ratio of acetonitrile to water at 70:30 (Figure 4).

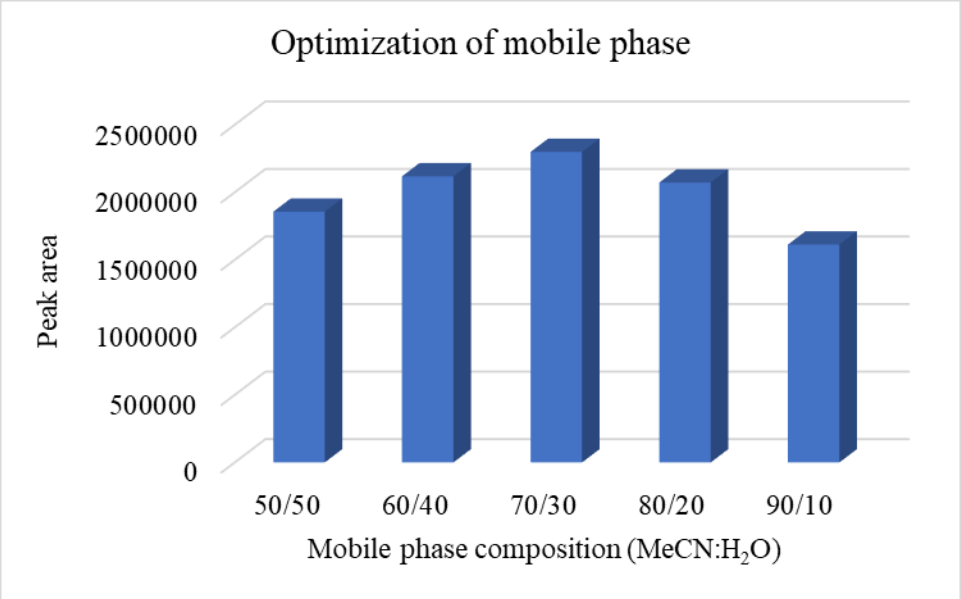

Figure 4. Optimization of mobile phase by peak area at 20 mg/L standard.

Detecting pesticides in the sample involved comparing the retention times (Rt) and PDA spectra of their peaks in the samples and those obtained from the injection of 20 g/L standards with mobile phase ACN/Water artio at 70:30%. The results indicate that the retention time for imidacloprid was 2.71 minutes. The duration of the analysis was approximately 5.0 minutes. This method is both cost-effective and environmentally friendly, as it requires only a small amount of organic solvent due to its short run time.

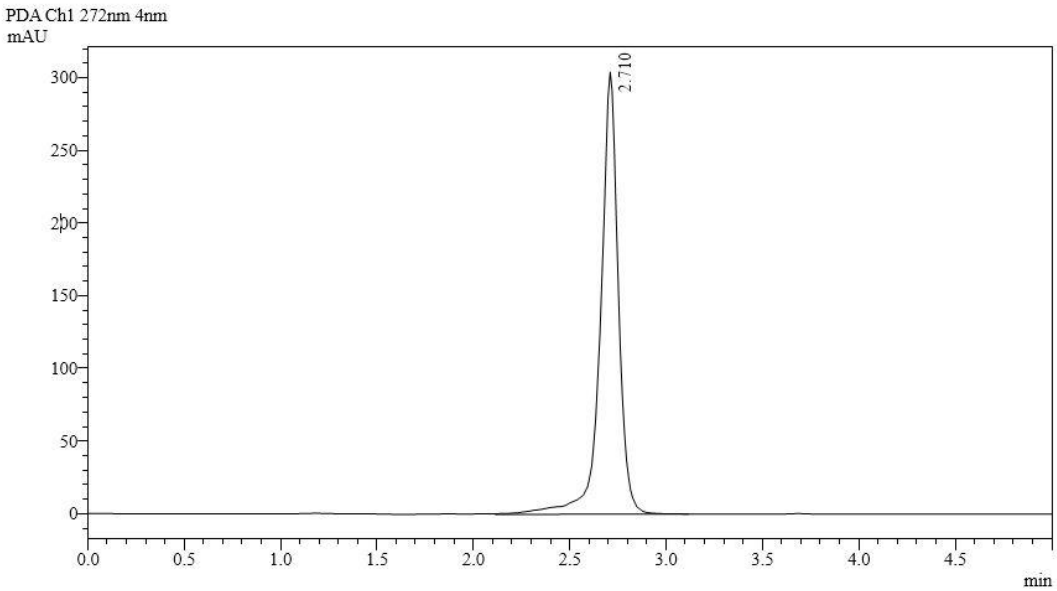

Figure 5. Chromatogram of imidacloprid standard of 20 mg/L.

Excellent chromatographic conditions were attained through isocratic elution, using a mobile phase of acetonitrile and water in a ratio of 70:30 (v/v), with a flow rate of 0.7 mL/min. The column temperature was maintained at a constant 30°C, and detection was performed via UV at a wavelength of 272 nm. The experimental conditions generated a consistent baseline, and the chromatographic peak of imidacloprid showed high, narrow, and symmetrical characteristics.

4.2. Validation of the method

The validation of the developed method was conducted to determine its suitability for future application. The validation process involved testing the method's specificity, selectivity, linearity, accuracy, and precision.

UV-photodiode array detection was used to assess the peak purity and analyte peak identity to confirm the specificity and selectivity of the developed method. The method's specificity and selectivity were evaluated by identifying the peak of interest and determining the index of peak purity. The chromatographic peak of imidacloprid was unaffected by any other compound, as indicated by a purity index value exceeding 999. Furthermore, the analyte was identified using the retention time value and match factor obtained through the overlapping of spectra from a pure analytical standard and absorption spectra of imidacloprid present in the pesticide formulation sample. The peak purity of imidacloprid standard are presented in Figure X. There were no other coeluted peaks that interfered with the chromatographic peaks of the active components, as observed in the pesticide formulation chromatograms. Thus, the resultant match factor value of 999.98 substantiated the identification of imidacloprid.

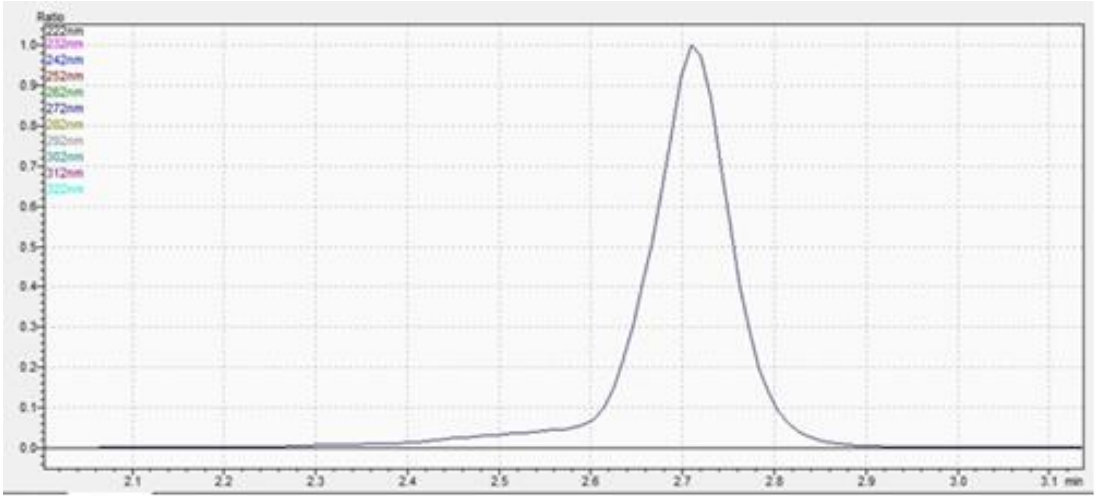

Figure 6. Peak purity spectra index by imidacloprid standard at retention time 2.71 minutes shows the selectivity of the method.

The chromatographic conditions were optimised to achieve excellent resolution, enhance the analyte's signal, and reduce the noise level. Figure X shows typical UHPLC-PDA chromatograms of standard, collected samples. No interference peaks were observed in the area of the retention times of the analytes, and the compound's analysis time was under 5.0 minutes. The specific retention time of the standards and analysed samples at 2.71 min without no interference peaks shoed good specificity and selectivity of the method.

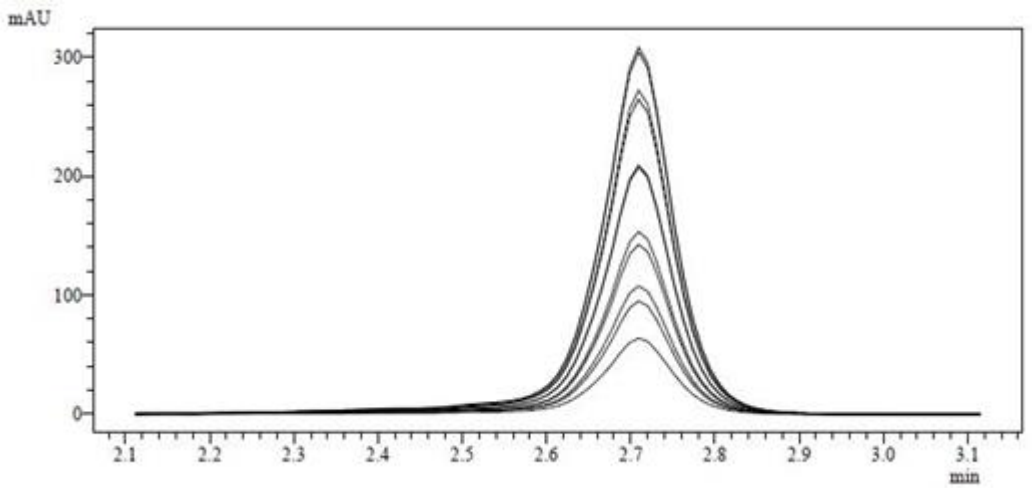

Figure 7. Peak profile of imidacloprid standards and analysed samples at retention times 2.71 min shows the specificity of the meyhod

The method's linearity was assessed by generating calibration curves that show the correlation between the injected amount of the analyte and the resulting peak area. A series of 5 working solutions in the 5.0-25.0 mg/L concentration range was constructed for this purpose. The table displays that the multiple correlation coefficients had values of R2 ≥ 0.9994. The results show that the proposed method showed excellent linearity (y=115463x - 2496.5) over the concentration range studied.

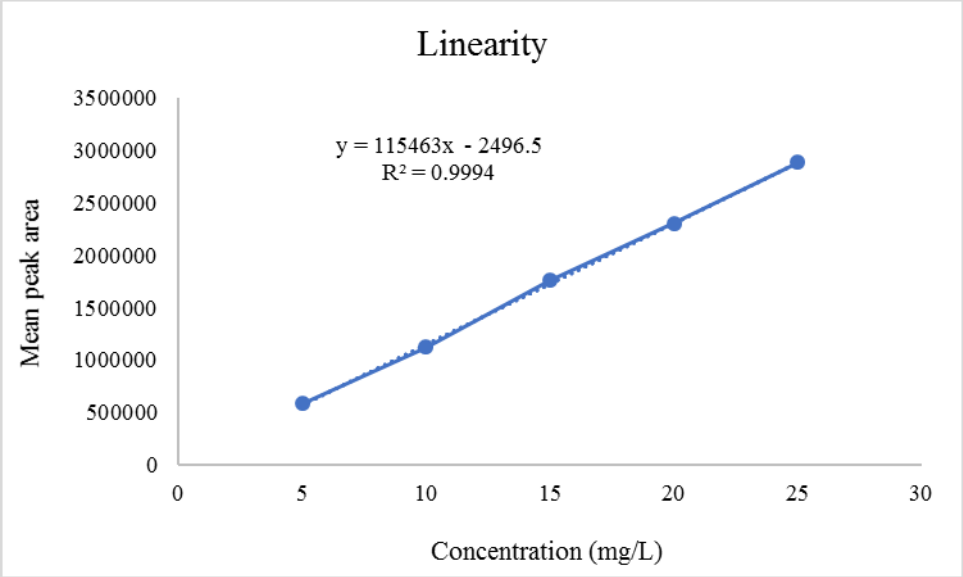

Figure 8. Standard curve of the method showed linearity of imidacloprid.

The method's accuracy was calculated as the difference between the calculated mean value obtained from UHPLC analysis and the true value of the analyte spiked into a sample matrix. The accuracy of the method also represnts as percentage of recovry of the true analyte present in the sample matrix. Precision was defined as the reproducibility of obtained data for imidacloprid retention time and peak area from three replicated injections for accuracy tests. The relative standard deviation (RSD) represnts the precision of the methods. The results of accuracy and precision of the method showed good recovery (98.9 % - 101.2 %) range at 3 spiked level with excellent pricision (lower than 1.89 %). Thus, it was concluded that the suggested method showed enough accuracy and precision to determine the active ingredients in the imidacloprid formulations.

Table 2. Accuracy of precision of the imidacloprid determination method.

Spiked amount (mg/L)	Accuracy (% recovery)	Precision (% RSD)
5	98.9	1.89
10	99.80	1.17
15	101.20	0.89

4.3. Purity analysis of collected imidacloprid samples

Ultrahigh-performance liquid chromatography with photodiode array detection (UHPLC-PDA) was used to analyze twenty one samples of seven commercially available Imidacloprid brands. Table 3 presents a summary of the purity analysis outcomes for the formulated brands.

Table 3. Percentage of active Ingredient present in some marketed brands of Imidacloprid 20 SL.

	Code	Formulation type	% purity	% mean purity
IDC-F1	R1	20 SL	125.94	125.94
	R2		125.4	
	R3		126.5	
IDC-F2	R1	20 SL	112.04	112.24
	R2		112.23	
	R3		112.45	
IDC-F3	R1	20 SL	113.62	113.39
	R2		113.33	
	R3		113.23	
IDC-F4	R1	20 SL	115.69	115.56
	R2		115.11	
	R3		115.87	
IDC-F5	R1	20 SL	118.91	118.67
	R2		118.43	
	R3		118.66	
IDC-F6	R1	20 SL	83.11	83.14
	R2		83.03	
	R3		83.28	
IDC-F7	R1	20 SL	2.91	2.92
	R2		2.86	
	R3		2.98	

The findings indicate that among the seven analyzed brands, one showed AI content that exceeded 125%. Four tested brands contained 110-120% of the active ingredient. One of the brands in the study exhibited a concentration of 80-90% of the necessary active ingredient. The other brand was found to have an AI content of less than 5%.

From the above results none of the imidacloprid brands contained standard active ingredients. The results showed mean purity of the brand IDC-F1, IDC-F2, IDC-F3, IDC-F4, IDC-F5 was over standard, while the brand IDC-F6 was substandard. The brands IDC-F7 had almost no active ingredients.

Figure 9-15 shows the chromatograms of the injected extracts of Imidacloprid pesticide samples containing detected purity.

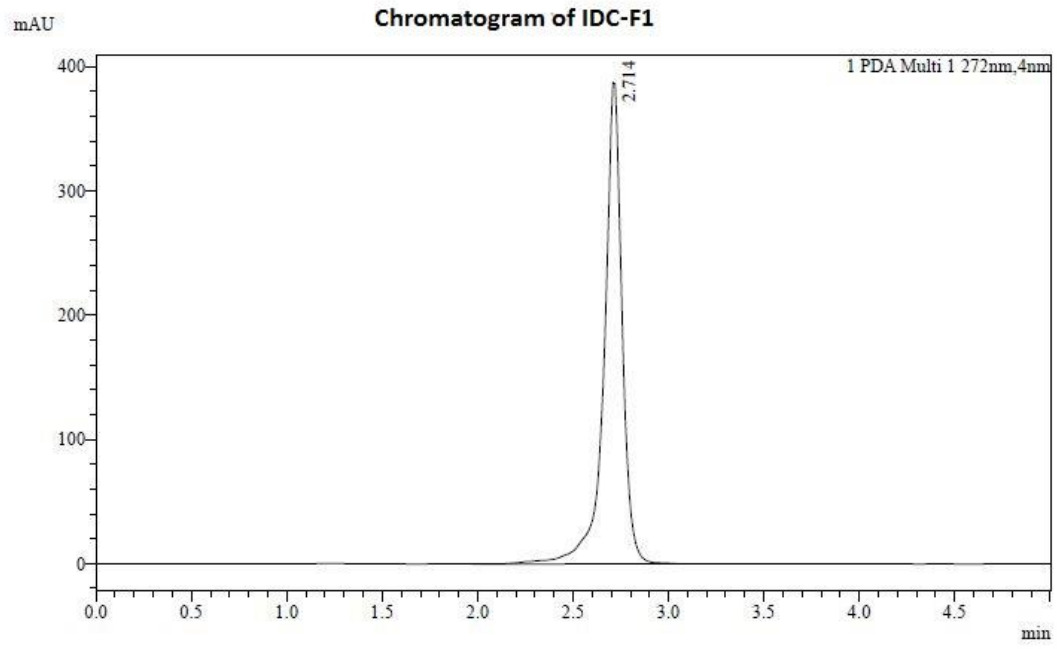

Figure 9. Chromatogram of imidacloprid found in the sample IDC-F1.

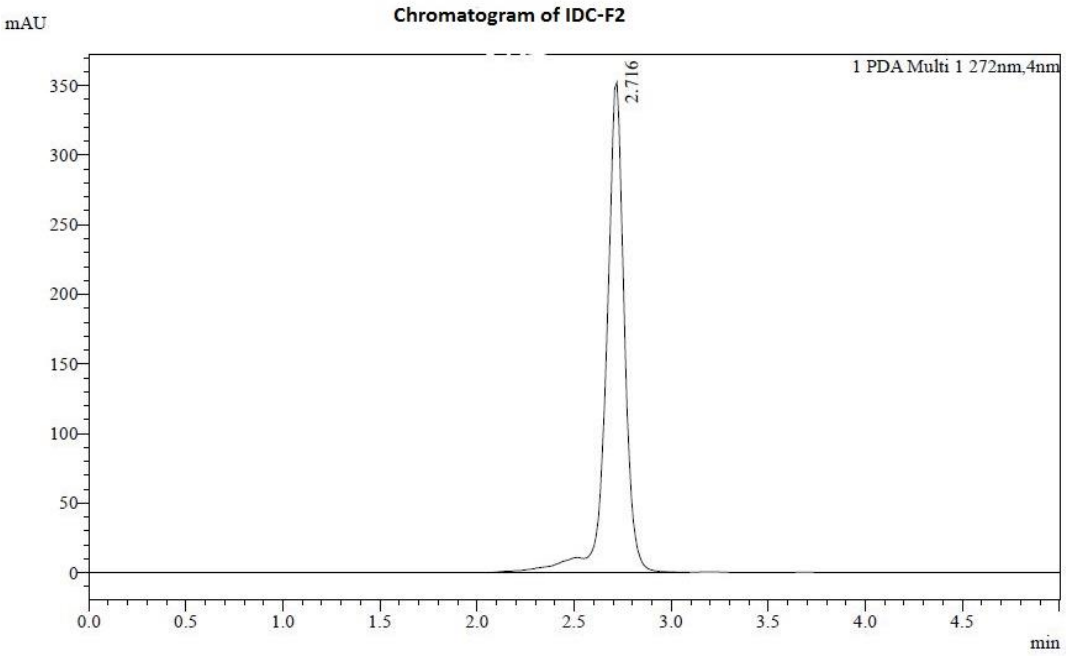

Figure 10. Chromatogram of imidacloprid found in the sample IDC-F2

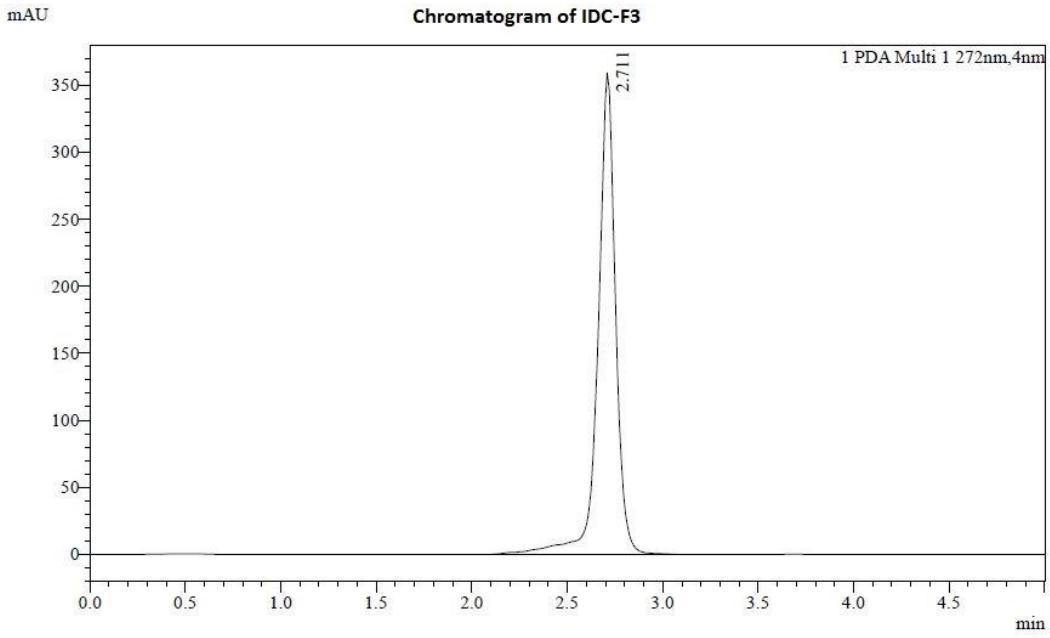

Figure 11. Chromatogram of imidacloprid found in the sample IDC-F3.

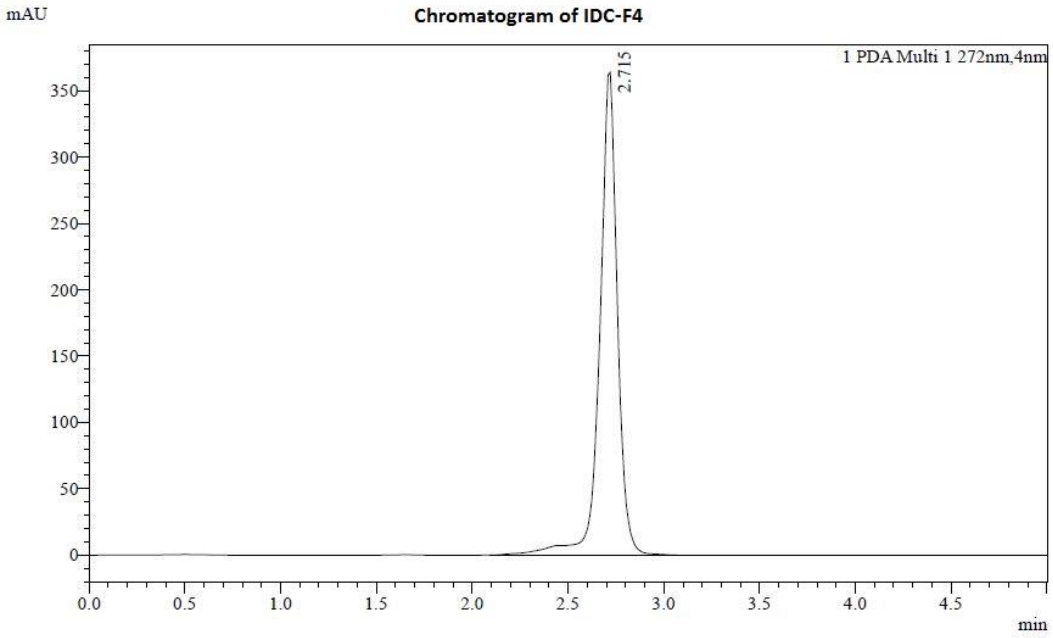

Figure 12. Chromatogram of imidacloprid found in the sample IDC-F4.

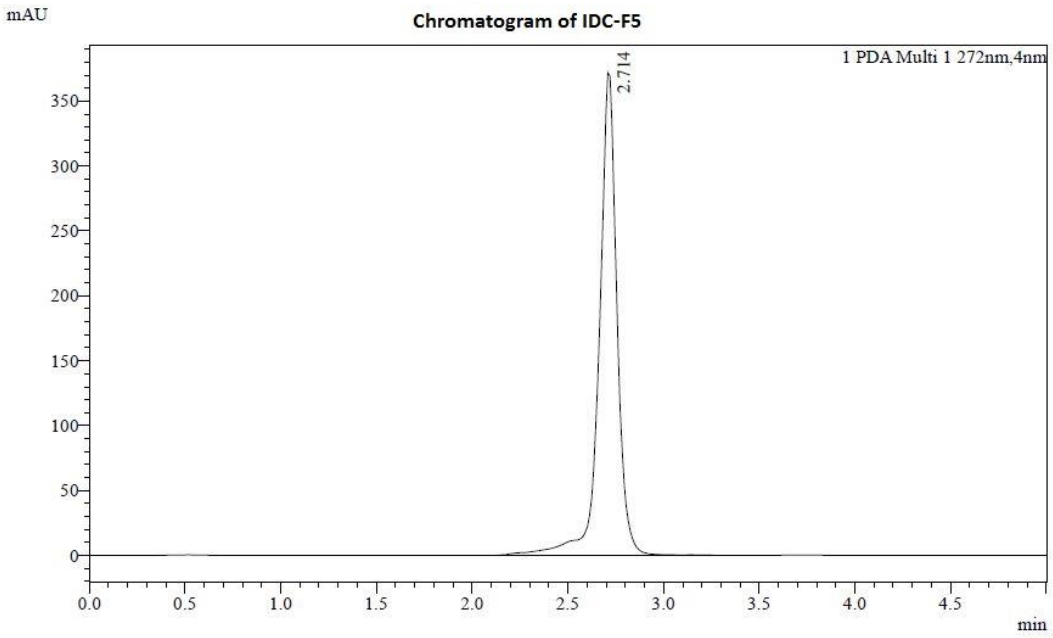

Figure 13. Chromatogram of imidacloprid found in the sample IDC-F5.

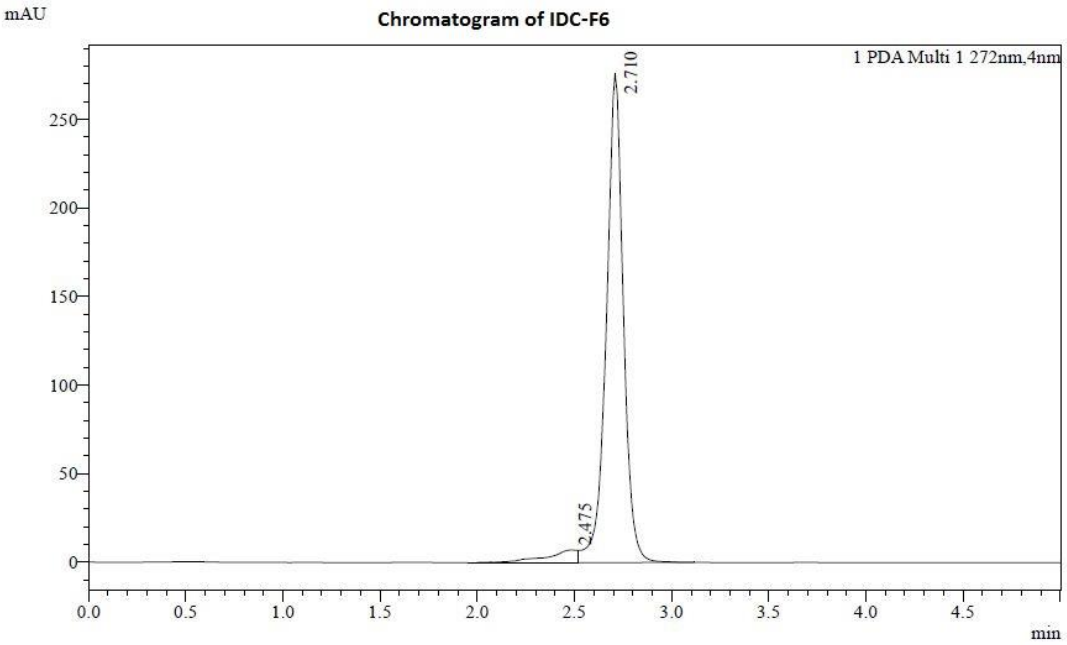

Figure 14. Chromatogram of imidacloprid found in the sample IDC-F6.

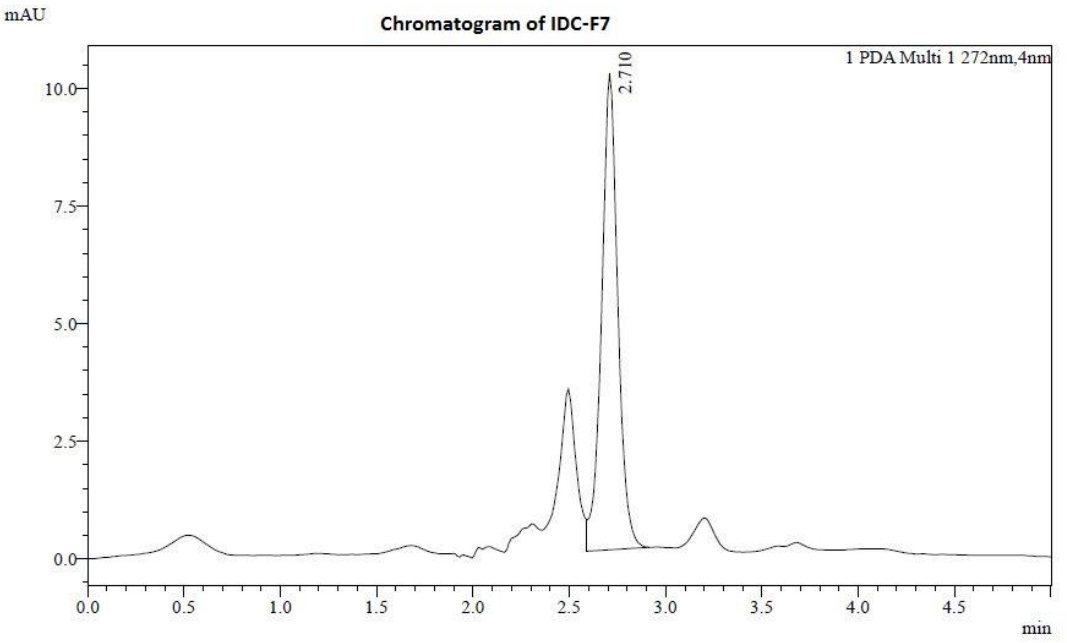

Figure 15. Chromatogram of imidacloprid found in the sample IDC-F7.

These results of marketed imidacloprid brands are unacceptable in contest of required AI presence. The reported results from commercially available imidacloprid formulations are deemed unsatisfactory when evaluated in the context of the stated active ingredient concentration.

Similar experiment also carried out by Fuad Al-Rimawi in 2014 (Fuad, 2014). He has been developed a simple, accurate, precise, and selective HPLC method and validated for determination of three pesticides (β-cyfluthrin, abamectin, and imidacloprid) in groundwater. The method is curate in determination of these pesticides with a wide dynamic range (1-1000 ppb for abamectin, 0.5-1000 ppb for imidacloprid, 0.4-1000 ppb for ß-cyfluthrin) with recovery from 97.6 to 101.5%. Low LOD and LOQ of the pesticides analyzed in this study enable the detection and quantitation of them in water at low concentrations. This validated method can be employed for the determination of these pesticides in real water samples, including groundwater, surfacewater and wastewater which were similar to imidaclorpid validation analysis.

CHAPTER V
SUMMARY AND CONCLUSION

The quality control of plant protection products is of the utmost importance in ensuring their efficacy and proper use, as counterfeit products have the potential to result in crop losses, endanger public health, and pose a threat to the environment and food trade. The assessment and measurement of the degree of AI purity are increasingly important in regulating the composition of formulated plant protection products, as any form of adulteration can adversely impact their quality, stability, and safety. The control of active compounds and their impurities in pesticide formulations requires reliable and comparable analytical results, which can be obtained by applying validated methods and implementing an effective quality assurance and quality control (QA/QC) system in the laboratory.

This study optimized and validated a new method for identifying and quantifying the active component imidacloprid in 20SL pesticides using a reversed-phase UHPLC-PDA technique with a Waters Cortecs C18 column (150 x 4.6 mm, 2.7 µm) column and isocratic elution with a mobile phase consisted of acetonitrile–water (70:30, v/v), a flow rate of 0.5 mL/min, a constant column temperature at 30 °C, and UV detection at 272 nm. The method is environmentally friendly and cost-effective due to it requires a small volume of organic solvent. The validated method showed excellent retention time and peak area repeatability and a high value for the multiple correlation coefficient in the linear calibration equation. The optimized and validated method was simple, quick, precise, and accurate for routine testing of the active component imidacloprid in pesticide formulations.

The purity of seven marketed brands of imidacloprid insecticide was found to vary based on analytical results. The analysis revealed that five distinct imidacloprid insecticide brands exhibited purity levels exceeding the established standard range of 110-130%. One of the brands of the imidacloprid insecticide available in the market was found to be substandard or impure in terms of quality. The imidacloprid sample exhibited poor purity, with a less than 90% purity level. The other brand was completely impure and had almost no active ingredient (less than 10%) of

imidacloprid. These amounts are not acceptable and don't meet standards. The present study concludes that the substandard quality of pesticide products may be attributed to adulteration resulting from inadequate quality control and quality assurance procedures in the pesticide production industry.

CHAPTER VI

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APPENDICES






Appendix 1: Different imidacloprid sample collection, preparation and analysis