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Pesticide Residue Analysis in Tea Using LC-MS/MS– QuEChERS Technique

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Abstract

Tea, the dried leaves of the plant of *Camellia sinensis* (L.) Kuntze, Theaceae, is an aromatic beverage that is consumed worldwide. Due to its properties of antioxidant, antimicrobial, anticarcinogenic and anti-inflammatory, tea has attracted great attention. However, tea farming is sensitive to many kinds of diseases, pests and weeds, which causes the widely use of pesticides. Until now, more than 300 kinds of pesticide residues in tea have been. Massive pesticide exposure can lead to pesticide accumulation in the body, which harms human health and causes various diseases, thus many countries have established maximum residue limits for many pesticides. Meanwhile, tremendous efforts have been performed in order to develop analytical methods for pesticides determination in tea. Hence this research focuses to establish High Performance Liquid Chromatography (HPLC) method for determining the residues of pesticides in tea.

Keywords: Tea, Pesticide residues, LC-MS/MS, QuEChERS

Introduction

Tea, derived from the dried leaves of Camellia sinensis, is cherished worldwide for its antioxidant, antimicrobial, anticarcinogenic, and anti-inflammatory properties. 70% of India's tea is consumed domestically, the nation is still among the top producers of tea. Assam is one of the leading states in India in terms of production. However, the widespread use of Pesticides in tea farming to combat diseases, pests, and weeds raises concerns about pesticide residues in the final product. Over 300 pesticide residues have been detected in tea, necessitating stringent regulatory measures to safeguard consumer health.

According to Food and Agriculture Organization (FAO) pesticides are any substance or combination of substances expected for forestalling, annihilating or controlling any vermin, including vectors of human and animal sicknesses, undesirable types of plants and creatures causing harm during or in any case meddling with the production, handling, stockpiling, transport or advertising of food, agrarian wares, wood and wood items, and animal feedstuffs, or substances that might be administered to animals for the management of insects, or different irritations in or on their bodies [1]. The term "pesticides" refers to "chemical agents used to prevent, eradicate, repel or neutralize any pest, ranging from microorganisms (i.e., algicides, fungicides or bactericides) to insects, rodents, and weeds (i.e., herbicides) [2].

Using pesticides is a key agricultural technique to increase food production in order to keep up with the rapidly expanding population and avoid vector-borne illnesses. Therefore, it is inevitable that pesticides would have an impact on the environment and that they will accumulate in the food chain, especially in developing countries. Because organochlorines are lipophilic, they may linger in ecosystems for a considerable amount of time and reach the food chain, where they may accumulate in adipose tissue [3]. A commercial pesticide product's health impact could result from the active component, other compounds in the formulation, or from both. However, the active substance is typically the only subject of toxicological testing. The combined substances have negative consequences, as determined by epidemiological surveillance of pesticide health impacts. The International Agency for Research on Cancer (IARC), a component of the World Health Organization states that "probable human carcinogens" include "occupational exposures in spraying and application of non-arsenical insecticides." This class of non-arsenical compounds such as carbamates and organophosphates are widely used and comprises several active components found in hundreds of commercial formulations [2,4].

The use of banned pesticides in the production of tea raises severe concerns since they could be harmful to human health and the environment. Pesticides that have been banned or restricted because of their negative effects on non-target creatures, the environment, and human health are known as banned pesticides. Some of the banned pesticides being utilized in tea plantations are Endosulfan, DDT, Ethyl parathion, and Chlordane [5].

In order to reduce harm to humans or animals and for all related matters, the Insecticides Act, 1968 and the Insecticides Rules, 1971 control the import, registration process, production, sale, transport, distribution, and use of insecticides (pesticides) across India. Before being made accessible for use or sale, all insecticides (also known as pesticides) must go through the registration process with the Central Insecticides Board & Registration Committee (CIB & RC) [6]. Excessive pesticide residues in tea can pose health risks, including acute toxicity, chronic health issues such as cancer, endocrine disruption, and neurological effects. Therefore, regular monitoring and analysis of pesticide 8 residues in tea are crucial to ensure that the tea consumed is safe and within regulatory limits. Legal restrictions on the maximum quantity of pesticide residue that is permitted to be found in food products are known as Maximum Residue Levels, or MRLs. The Food Safety and Standards Authority of India (FSSAI) sets the maximum residual levels (MRLs) of pesticides in tea in India. Usually, mandatory analyses include checking tea for pesticides with known maximum residue levels [5].

The analysis of pesticide residues in tea involves several advanced techniques to detect and quantify the presence of these chemicals. Some of these techniques are Gas Chromatography, Liquid Chromatography, and ELISA. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) is a sample preparation technique that simplifies the extraction and clean-up process for pesticide residue analysis. Due to the relatively limited selectivity of analytical instrumentation, such as gas chromatography mass spectrometry and liquid chromatography mass spectrometry (GC–MS and LC–MS), the classical extraction methods require numerous steps. In contrast, GC and LC-tandem mass spectrometry (GC–MS/MS and LC– MS/MS) has been used more frequently with the QuEChERS method recently because of its high selectivity, sensitivity, and specificity [7].

LC-MS/MS is a popular choice because it can be used to handle more polar pesticides as well as pesticide metabolites, which are frequently less volatile and more polar than the pesticide itself. The majority of LC/MS/MS applications for pesticide analysis are concentrated on non-GC amenable chemicals, thermolabile, polar, and non-volatile pesticides. This technique is still becoming more and more common. Both GC/MS and LC/MS/MS can be used to analyse certain chemical classes, including phenoxyacids, herbicides, triazines, OPs, chloroacetanilides, and pyrethroids. LC/MS/MS is thought to be preferable for phenoxacid herbicides and carbamates since it eliminates the need for a derivatization step before analysis [8]. In the present study, a novel LC- MS/MS – QuEChERS technique was developed for the estimation of pesticides in tea for the first time.

Materials and Methods:

Chemicals and reagents

- Acetonitrile (Fisher Scientific)
- n-Hexane (Merck)
- Methanol (J.T. Baker)
- Sodium sulphate anhydrous (Qualigens)
- $MgSO_4$ (Qualigens)
- Sodium Chloride (Qualigens)
- PSA (Sigma Aldrich)
- Disodium citrate sesquihydrate (Sigma Aldrich)
- Tri sodium citrate (Rankem)
- GCB (Sigma Aldrich)

Instrument used

Agilent: LC: (G1312B) HPLC-1260 Binary pumps. MS MS: G6430A Triple Quad MS.

Preparation of standard solution

Stock Standards: The stock solutions of the individual pesticide standards were prepared by accurately weighing 10 mg of each analyte in volumetric flasks and dissolving in 10 ml Methanol/ Ethyl acetate. These were stored in dark vials in a refrigerator. The concentration of stock solution was 1000 µg/ml.

Intermediate Stock Standard Mixture I: A 10 µg/ml standard was prepared by adding a 0.1 ml individual stock standard solution to a 10 ml volumetric flask and diluting to the mark with Methanol/Ethyl acetate.

Working Standard Mixture: A 1 µg/ml standard was prepared by adding a 1.0 ml intermediate stock standard mixture to a 10ml volumetric flask and diluting to the mark with Methanol/ Ethyl acetate.

Linearity Range - 0.005, 0.01, 0.025, 0.05, 0.075, 0.100, 0.125 mg/k. Preparation of calibration curve standards: Mixture of Calibration Standards: Multimix in 5 to 125 ppb (Table 1).

Table 1: Multimix in 5-125 ppb

Stock concentration in ppb	Volume of Stock (µl)	Remaining Volume (µl)	Final Volume(ml)	Final conc. (ppb)	Label
1000	0	1000	1	0	STD-1
1000	5	995	1	5	STD-2
1000	10	990	1	10	STD-3
1000	25	975	1	25	STD-4
1000	50	950	1	50	STD-5
1000	75	925	1	75	STD-6
1000	100	900	1	100	STD-7
1000	125	875	1	125	STD-8

Procedure

Extraction of pesticides:

Two commercial tea samples were purchased from local markets in Chennai, India. 10.0 g of sample was taken and 10ml of acetonitrile and 5 ml of water was added to it. The mixture was vortexed and 1 g of magnesium sulphate, 0.5 g trisodium citrate dehydrate, 0.5 g disodium citrate sesquihydrate, and 1g of sodium chloride were added to it. It was shaken immediately and centrifuges at 5000 rpm for 5 mins at a temperature of 20˚C. 2ml of clear acetonitrile layer was taken for cleanup with 30 mg of Prostate-Specific Antigen, 20 mg of magnesium sulphate and 15 mg of graphitized carbon black. This was vortexed and centrifuged at 5000 rpm for 5 minutes at a temperature of 20˚C. From this, 1 ml of clear solution was taken out and placed in GC-MS/MS. 1ml was pipetted out and the extract was evaporated using Turbo evaporator at 40±2˚C under nitrogen. The residue was reconstituted with 1ml of water and injected to LC-MS/MS.

Instrument conditions for LC-MS/MS:

HPLC Conditions:

The LC-program for the Pesticide compounds. Eclipse plus C18 column 150 mm x 4.6, 3.5 µm (Agilent technologies) was used with Methanol and 0.1 % Formic acid in gradient. The flow rate was 0.400 ml/min. The column was kept 40˚C. The injection volume was 25 micro liter and run time 25 min.

The liquid chromatography elution table is given in (Table 2).

LC-MS-MS Conditions:

Selected reaction monitoring MS/MS was performed on the protonated molecular ion for pesticides using the general parameters as given in (Table 3).

Polarity: Positive & Negative.
Ion Source: Electro Spray

Table 2: Liquid chromatography elution method

	Channel	Solvent 1	Name 1	Used	Percent
1	A	H20	0.1 % FA	Yes	90.0 %
2	B	MeOH		Yes	10.0 %
	Time	A	B	Flow	Pressure
1	3.00 min	90.0 %	10.0 %	0.400 mL/min	600.00 bar
2	6.00 min	5.0 %	95.0 %	0.400 mL/min	600.00 bar
3	13.00 min	5.0 %	95.0 %	0.400 mL/min	600.00 bar
4	13.10 min	90.0 %	10.0 %	0.400 mL/min	600.00 bar
5	25.00 min	90.0 %	10.0 %	0.400 mL/min	600.00 bar

Table 3: Source Parameters

Parameter	Value (+)	Value (-)
Gas Temperature (°C)	250	250
Gas Flow (1/min)	8	8
Nebulizer (psi)	45	45
Capillary (V)	4000	4000

Results and Discussion

It is quite difficult to quantify pesticides simultaneously in a single technique analysis when considering ionization, sample extraction, appropriateness, potential pesticides, and chromatography. However, the LC/MS/MS technique utilized is more sophisticated and sensitive. Quantification of pesticides was performed by LC-MS/MS method for two commercial tea samples that were purchased from local markets in Chennai. Tables 4 and 5 provides the quantities of pesticides found in Sample 1 and 2.

The concentration of the pesticides in the samples 1 and 2 were found to be below the limit of quantification (BLQ). The selected reaction monitoring conditions are shown in the Multiple Reaction Monitoring (MRM) Parameters. For every component, the usual product ion produced in these circumstances, the MRM transition for pesticides is given in the (Table 6).

Table 4 : The quantities of pesticides found in Sample 1

S. No.	Test Parameters	Units	Results
1	2,4-D	mg/kg	BLQ (LOQ:0.01)
2	Bifenthrin	mg/kg	BLQ (LOQ:0.01)
3	Bitertanol	mg/kg	BLQ (LOQ:0.01)
4	Carbendazim	mg/kg	BLQ (LOQ:0.01)
5	Carfentrazone Ethyl	mg/kg	BLQ (LOQ:0.01)
6	Chlorpyrifos	mg/kg	BLQ (LOQ:0.01)
7	Chlothianidin	mg/kg	BLQ (LOQ:0.01)
8	Cyflumetofen	mg/kg	BLQ (LOQ:0.01)
9	Deltamethrin	mg/kg	BLQ (LOQ:0.01)
10	Dicofol (sum of o,p and p,p'isomers)	mg/kg	BLQ (LOQ:0.01)
11	Emamectin Benzoate	mg/kg	BLQ (LOQ:0.01)
12	Ethion	mg/kg	BLQ (LOQ:0.01)
13	Etoxazole	mg/kg	BLQ (LOQ:0.01)
14	Fenazaquin	mg/kg	BLQ (LOQ:0.01)
15	Fenpropathrin	mg/kg	BLQ (LOQ:0.01)
16	Fenpyroximate	mg/kg	BLQ (LOQ:0.01)
17	Flubendiamide	mg/kg	BLQ (LOQ:0.01)
18	Fluvalinate	mg/kg	BLQ (LOQ:0.01)
19	Glufosinate Ammonia	mg/kg	BLQ (LOQ:0.01)
20	Glyphosate	mg/kg	BLQ (LOQ:0.01)
21	Hexaconazole	mg/kg	BLQ (LOQ:0.01)
22	Hexythiazox	mg/kg	BLQ (LOQ:0.01)

23	Lambda Cyhalothrin	mg/kg	BLQ (LOQ:0.01)
24	Mancozeb as CS2	mg/kg	BLQ (LOQ:0.01)
25	Oxyfluorfen	mg/kg	BLQ (LOQ:0.01)
26	Paraquat	mg/kg	BLQ (LOQ:0.01)
27	Propargite	mg/kg	BLQ (LOQ:0.01)
28	Propiconazole	mg/kg	BLQ (LOQ:0.01)
29	Quinalphos	mg/kg	BLQ (LOQ:0.01)
30	Spiromesifen	mg/kg	BLQ (LOQ:0.01)
31	Thiacloprid	mg/kg	BLQ (LOQ:0.01)
32	Thiamethoxam	mg/kg	BLQ (LOQ:0.01)
33	Zineb as CS2	mg/kg	BLQ (LOQ:0.01)

Table 5: The quantities of pesticides found in Sample 2

S.No.	Test Parameters	Units	Results
1	2,4-D	mg/kg	BLQ (LOQ:0.01)
2	Bifenthrin	mg/kg	BLQ (LOQ:0.01)
3	Bitertanol	mg/kg	BLQ (LOQ:0.01)
4	Carbendazim	mg/kg	BLQ (LOQ:0.01)
5	Carfentrazone Ethyl	mg/kg	BLQ (LOQ:0.01)
6	Chlorpyrifos	mg/kg	BLQ (LOQ:0.01)
7	Chlothianidin	mg/kg	BLQ (LOQ:0.01)
8	Cyflumetofen	mg/kg	BLQ (LOQ:0.01)
9	Deltamethrin	mg/kg	BLQ (LOQ:0.01)
10	Dicofol (sum of o,p and p,p'isomers)	mg/kg	BLQ (LOQ:0.01)
11	Emamectin Benzoate	mg/kg	BLQ (LOQ:0.01)
12	Ethion	mg/kg	BLQ (LOQ:0.01)
13	Etoxazole	mg/kg	BLQ (LOQ:0.01)
14	Fenazaquin	mg/kg	BLQ (LOQ:0.01)
15	Fenpropathrin	mg/kg	BLQ (LOQ:0.01)
16	Fenpyroximate	mg/kg	BLQ (LOQ:0.01)
17	Flubendiamide	mg/kg	BLQ (LOQ:0.01)
18	Fluvalinate	mg/kg	BLQ (LOQ:0.01)
19	Glufosinate Ammonia	mg/kg	BLQ (LOQ:0.01)
20	Glyphosate	mg/kg	BLQ (LOQ:0.01)
21	Hexaconazole	mg/kg	BLQ (LOQ:0.01)
22	Hexythiazox	mg/kg	BLQ (LOQ:0.01)
23	Lambda Cyhalothrin	mg/kg	BLQ (LOQ:0.01)
24	Mancozeb as CS2	mg/kg	BLQ (LOQ:0.01)
25	Oxyfluorfen	mg/kg	BLQ (LOQ:0.01)
26	Paraquat	mg/kg	BLQ (LOQ:0.01)
27	Propargite	mg/kg	BLQ (LOQ:0.01)
28	Propiconazole	mg/kg	BLQ (LOQ:0.01)
29	Quinalphos	mg/kg	BLQ (LOQ:0.01)
30	Spiromesifen	mg/kg	BLQ (LOQ:0.01)
31	Thiacloprid	mg/kg	BLQ (LOQ:0.01)
32	Thiamethoxam	mg/kg	BLQ (LOQ:0.01)
33	Zineb as CS2	mg/kg	BLQ (LOQ:0.01)

Table 6: MRM Parameters

Compound Name	Precursor Ion	Prod Ion	Polarity
Emamectin Benzoate	887	158.2	Positive
Fluvalinate	503.14	443.6	Positive
Cyflumetofen	448.2	145.1	Positive
Fenpyroximate	422.2	206	Positive
Spiromesifen	371.2	273.3	Positive
Oxyfluorfen	362	167.6	Positive
Etoxazole	360.2	141	Positive
Hexythiazox	353.1	228.2	Positive
Propargite	351.2	189.3	Positive
Fenpropathrin	350.2	97.1	Positive
Chlorpyrifos	349.94	125.2	Positive
Propiconazole	342.08	159.1	Positive
Bitertanol	338.19	99.2	Positive
Hexaconazole	314.1	159	Positive
Fenazaquin	307.2	161.1	Positive
Quinalphos-f	299.1	167.9	Positive
Thiamethoxam	292.7	182	Positive
Thiacloprid	253.7	126.5	Positive
Carbendazim	192.1	160	Positive
Paraquat-1	186	77.1	Positive
Glufosinate ammonium	182.1	136.1	Positive
2,4-D	220.9	162.9	Negative
Glyphosate	168.1	149.9	Negative

Conclusion

The presence of pesticide residues in food samples poses a significant threat to public health. Ensuring the safety of food products, such as tea, requires rigorous testing for these residues. To evaluate the levels of residual pesticides in tea samples, the advanced analytical technique of Liquid Chromatography-Mass Spectroscopy (LC-MS/MS) is employed. In this study, the concentrations of 33 different pesticides were measured in two tea samples. The results indicated that the pesticide levels in both samples were below the limit of quantification (LOQ), suggesting minimal or no detectable pesticide residues. The findings of this analysis demonstrate the absence of detectable pesticide residues in the tested tea samples. Consequently, these tea samples can be deemed safe for human consumption, reducing the concern for potential adverse health effects associated with pesticide exposure.

Regular monitoring and analysis of pesticide residues in food products are essential to ensure food safety. This practice helps to prevent consumer exposure to harmful pesticides and protects public health. Continuous surveillance and stringent regulatory measures are necessary to maintain the safety and quality of food items available to consumers. In conclusion, the study highlights the critical importance of testing for pesticide residues in food products to safeguard public health. The use of sophisticated analytical techniques like LC-MS/MS provides accurate and reliable data on pesticide levels, ensuring that food items such as tea are safe for consumption. Regular monitoring is a key aspect of food safety management, helping to mitigate the risks associated with pesticide exposure and promoting overall public health.

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