International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2024; 12(5): 48-56

Received: 15-07-2024
Accepted: 10-08-2024

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The evaluation of pesticide exposure in cannabis using advanced analytical techniques: A review

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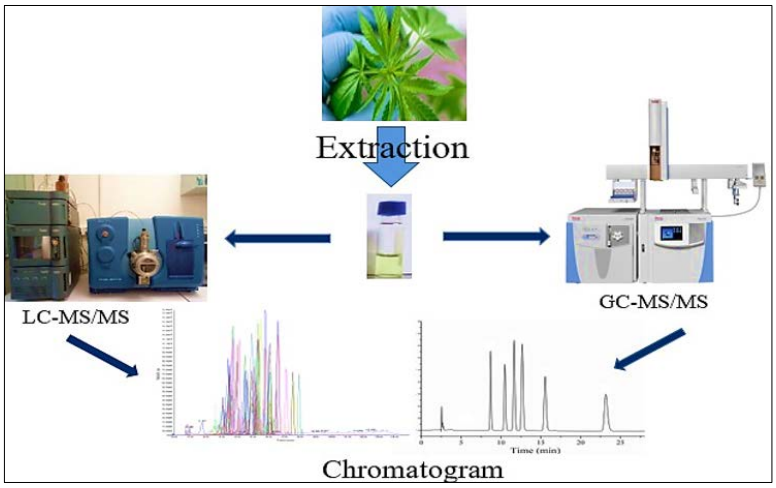
DOI: https://dx.doi.org/10.22271/chemi.2024.v12.i5a.12437

Abstract
Cannabis has received a lot of consideration in the last few years due to an increase in the number of countries that have legalized it for both recreational and medical purposes. The quality control checks and techniques for characterizing cannabis products have grown enormously along with the growing demand for safer cannabis cultivation as well as its safer products. Pesticide residue analysis in cannabis has gained increasing attention in recent years. Cannabis products should be tested for authorised and unauthorised pesticides to meet regulatory and quality control standards. This manuscript overviews Liquid-liquid extraction, solid-phase extraction, and QuEChERS sample preparation and clean-up procedures for pesticide extraction in cannabis products while mass spectrometry techniques are utilized for crucial identification and quantification. In this study, the benefits and drawbacks of the various analytical techniques utilized for pesticide residue analysis in cannabis and its products are assessed. Future directions and difficulties are also considered.



Graphical Abstract- A schematic representation of the process for the Analysis of pesticides in cannabis and its products using mass spectrometry techniques

Keywords: GC-MS/MS, LC-MS/MS, LLE, SPE, QuEChERS

Introduction
The terms "cannabis," "weed," "pot," and "marijuana" all refer to the same family of plants that have sedative and soothing properties [1]. However, depending on how you consume it, the consequences vary, and it's banned in many countries. The cannabis (*Cannabis Sativa*) contains chemicals called cannabinoids, including cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) [2]. Cannabis cannabinoids function by attaching to particular locations on the nerves and in the brain [3]. Although there are more than 100 cannabinoids in cannabis, THC and CBD have undergone the most research. The largest concentrations of cannabinoids are found in the plant's leaves and flowers.

The most widely utilized substance worldwide is cannabis. 145 nations reported regularly cultivating cannabis between 2010 and 2016. The most popular way to consume this medication is by smoking herbal cannabis. In the UK, cannabis is now classified as a class B restricted drug. The UK government was hesitant to legalize this substance for medicinal usage. Since November 1st, 2018, qualified medical professionals are permitted to write prescriptions for cannabis-based medications [4].

According to the 2017 World Drug Report, 183 million people worldwide consume cannabis every year. By the end of 2025, the worldwide legal cannabis industry is anticipated to be worth USD 147 billion [5]. The need for cannabis product safety has increased with the industry's rising demand. The regulatory bodies in the nations where cannabis use is allowed have established stringent rules to guarantee the efficacy, safety, and quality of cannabis products. Similar classes of pollutants are regulated by these rules, although the exact chemicals and their action limits may differ. In general, testing for pesticides, heavy metals, mycotoxins, and microorganisms is part of quality control for cannabis products; testing for residual solvents in cannabis oil is occasionally part of this process as well [6, 7]. Because consumers may be exposed to all of these toxins during the plant's growing or processing stages, it is important to keep an eye on them. The kinds of literature are well-researched on the toxicological consequences of pesticides, including their teratogenicity, neurotoxicity, and carcinogenicity [8, 9, 10].

Pesticides, mostly insecticides and herbicides are frequently administered consistently to cannabis plants grown for human use to eliminate undesired pests [11, 12]. Even while pesticide usage has advantages for agriculture, there are several problems associated with it, including environmental issues including pollution of the land, water, and air, impacts on natural systems, and negative effects on human health [13].

For instance, it has been discovered that the organophosphorus pesticide and insecticide chlorpyrifos are very toxic and have effects on the neurological system. The pesticide dichlorodiphenyltrichloroethane (DDT), which has been outlawed in many nations, is another example of an endocrine disruptor having effects on the cardiovascular, metabolic, and reproductive systems of humans [14, 15].

The preparation of samples at the trace level for residue analysis is extremely important in analytical chemistry, but it is also quite challenging. Analyzing analytes is a difficult undertaking, especially for cannabis leaf products and samples because of their complexity [16]. As a result, sample preparation is a necessary first step before instrumental analysis to design a method for quantification. Typically, it takes the longest in the entire experimental study, thus the accuracy and precision of the result should be optimized and assessed before using a certain approach [17].

The traditional methods, such as solid phase extraction (SPE) [18] and liquid-liquid extraction (LLE) [19], are thorough yet offer high extraction efficiency and sample cleaning. These traditional methods have drawbacks including the extensive use of risky organic solvents and the drawn-out extraction process. In addition to these limitations, particularly in LLE, the development of an emulsion and the acquisition of a restricted preconcentration are critical elements determining the accuracy of the approach and the effectiveness of the extraction. Therefore, in addition to these restrictions, ensuring environmental friendliness was a top priority, which shifted the attention to microextraction techniques as a replacement for traditional procedures [20].

Mass spectrometry (MS) is the preferred detection method for this application, according to the EU standards on analytical quality control and method validation processes for the measurement of pesticide residues [21]. However, alternative detection systems may also be used. The fundamental justification for this is that MS gives the identification of compounds more assurance. While gas chromatography (GC) and MS have historically been used to identify various classes of pesticides, liquid chromatography (LC-MS) is currently more frequently employed in this capacity [22]. For the investigation of a wider variety of chemicals, particularly for more polar pesticides and pesticide metabolites, as well as for non-volatile analytes, LC-MS is more appropriate [23]. The majority of GC studies of cannabis employ low polarity phase stationary phases, primarily 5% diphenyl and 95% dimethyl polysiloxane, from which these chemicals elute at temperatures below 300 °C in a brief analysis period (often less than 30 min). FID and MS detectors are the most often utilized types. Both detectors are mentioned in the 2009 United Nations guidelines for the detection and analysis of cannabis and cannabis products [24].

Because derivatization for GC analysis and the decarboxylation of relevant precursory compounds require additional sample preparation procedures, cannabis testing laboratories often prefer the use of LC for cannabinoid detection [25, 26].

This review article addresses the procedures used in the area of cannabis pesticide analysis for sample preparation, cleanup, and analysis. Its goal is to highlight notable and illustrative instances of analytical procedures for the determination of pesticide residue in cannabis, rather than to be entirely complete. Discussions focus in particular on more modern analytical techniques and historical instances. Although the processes covered above go into depth on the analysis of pesticide residues, they may also be used to build methods for the detection of other analytes in cannabis samples, which is useful for the developing area of cannabis chemistry.

Regulations of pesticide residues for cannabis and its products

Cannabis and cannabinoids' pharmacological characteristics have attracted a lot of attention in recent years from researchers. Cannabis and cannabinoids have been investigated for their potential therapeutic benefits in treating PTSD, neurological dysfunctional symptoms, and pain. Currently, one cannabis-derived drug product and three cannabis-related drug products have received FDA approval from the United States [27]. Synthetic cannabinoids that have received FDA approval are given specifically for the treatment of anorexia nervosa and chemotherapy-related nausea. Many patients also choose medicinal cannabis, which is allowed in more than half of the states in the United States and may be easier to obtain than prescription medications. However, unlike other prescription drugs, medicinal cannabis has not gone through the FDA clearance process in the United States and is not subject to the same supply chain rules [28].

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) governs the use of pesticides in agricultural products (U.S. Government, 1996) [29, 30]. But because cannabis is classified as a Schedule I drug by the federal government (US Drug Enforcement Administration, 2021), the US Environmental Protection Agency (US EPA) has not yet published any guidelines on the use of pesticides on cannabis [31]. The general public anticipates that as a result of the wave

of legalization of cannabis for medical or recreational use across the U.S., there will be legislation to assure the safety of cannabis use [32].

Sample preparation methodologies

During analysis, it can frequently be difficult to isolate analytes for instrument detection with high sensitivity, precision, accuracy, and specificity [33]. This is particularly true of items connected to cannabis because their chemical makeup can vary greatly [34]. The strong matrix effect and different matrices in different cannabis-related goods provide one of the main difficulties in developing analytical methods for the identification of pesticide residues in distinct cannabis products [35]. Samples often need strict sample preparation procedures, which may be broken down into four parts, to reduce this.

a. To increase the effectiveness of extraction, the cannabis plant material should be homogenized.
b. To minimize matrix interference during analysis, a sample clean-up technique and an appropriate extraction method should be used
c. The sample is pre-concentrated to increase sensitivity, accuracy, and precision.
d. The sample is stabilised by reconstituting in an appropriate inert solvent.

When using multi-residue techniques, sample preparation and clean-up a crucial steps in obtaining the greatest number of compounds in a single step. Since the type of matrix used in cannabis products is complicated [36]. The extraction of pollutants can be adversely affected by any element contained in related matrices like oils, hemp, or foods, leading to substantial matrix impacts. The study of pesticide residues in cannabis-related goods has been reported to involve a range of different sample-cleaning techniques [37]. Solid-liquid extraction (SLE) and QuEChERS (rapid, simple, cheap, effective, rugged, and safe) were the two most popular extraction techniques created for removing dangerous residues and pollutants (pesticides) from cannabis products [38, 39].

Liquid-Liquid extraction

Liquid-liquid extraction was traditionally considered to be a standard pre-concentration and matrix segregation procedure in analytical chemistry. It is a particular type of separation technique based on the "partitioning" phenomenon, in which one solute phase is transferred from one solvent to the other. Both solvents are immiscible or partly or semi-miscible within themselves. In most cases, one of the solvents is an aqueous medium (water) and the other is an organic non-polar liquid. Two steps make up LLE: the first is mixing (contacting), and the second is phase separation [40].

It is crucial to take into account both the procedures for choosing the right solvent and for controlling the mode of operation. It is advised to vigorously mix the two phases simultaneously to improve the transfer of desired analytes from one solvent to the other. Additionally, this aids in the development of emulsification, which makes phase separation easier. Practically, a "distribution coefficient" (or "K") rule is adhered to. When the chemical potential of the extractable solute is consistent across both phases, we may conclude that equilibrium has been reached.

Recently to analyze the 66 pesticides and 5 mycotoxins mandated by the State of California in cannabis tinctures made from medium-chain triglyceride (MCT) oil, a unique LC /MS/ MS technique was developed using a dual ESI and APCI source. The LOQs of all pesticides and mycotoxins in non-inhalable or edible cannabis-based products were well below the California action limits for these analytes. The remaining 4 pesticides were identified using LC /MS/ MS with an APCI source, while 62 of the 66 pesticides and 5 mycotoxins were analyzed using LC /MS/ MS with an ESI source. [41].

Solid phase extraction

A popular method for sample preparation and pre-treatment, particularly for trace analytes, is solid-phase extraction (SPE). It uses less solvent and has a higher recovery than other extraction methods, making it robust enough to perform an efficient preconcentration [42]. A key component of the SPE process is choosing the appropriate solvent and sorbent. The variety of sorbents for SPE ranges from graphitized carbon to chemically bound C8, C18, and various types of silica, among other materials. SPE provides a lot of benefits, but it also has some drawbacks, such as a high price and batch-to-batch volatility that impairs reproducibility [43].

The fundamental idea underlying SPE is that it is a technique for partitioning the liquid phase from the solid sorbent phase. The chemicals that dissolve in liquid mixtures are separated from the other compounds based on their chemical and physical characteristics using this sample preconcentration technique [44]. Two stages are involved: Using mobile phases, SPE separates the mixture into the wanted and non-desired components by using the affinity of the dissolved solutes in the liquid for the solid through which the samples are permitted to pass (stationary phase). The sample's analytes are allowed to pass through the stationary phase before being either collected or rejected, depending on whether they contain the analytes we are looking for.

For the GC-MS detection of four high-priority pesticides (atrazine, terbuthylazine, acetochlor, and alachlor) in a cannabis bud sample matrix, a molecularly imprinted solid-phase extraction (MISPE) method was created. The ideal elution fraction included 70% (v/v) methanol, whereas the ideal wash fraction contained 20% (v/v) methanol in an aqueous solution. Comparative investigations employing a synthetic non-imprinted polymer and a commercial C18 cartridge as reference sorbents allowed researchers to confirm the selectivity, accuracy, and recovery of the MISPEs. The maximum residue limit (MRL) was spiked into the cannabis bud sample in the amount of 3 g at a concentration of 0.05 mg/kg. When using the optimized MISPE procedures, the recovery of the four chosen pesticides recovered from the spiked samples was between 76.4 and 85.0%, as opposed to 91.6 to 96.9% for the C18 SPE [45].

A technique was developed using a gas chromatograph in conjunction with a high-resolution, precision mass quadrupole time-of-flight (GC/Q-TOF) mass spectrometer to screen cannabis extracts for more than 1,000 pesticides and environmental contaminants. Acetonitrile and solid-phase extraction purification were used for the extraction process. Extracts were diluted 125:1 with solvent before analysis. Two cannabis samples had carbaryl and malathion concentrations 4,000- and 10-fold higher, respectively than the maximum residue levels established by Canada and the U.S. Environmental Protection Agency for dried cannabis flowers [46].

Solid phase micro extraction: In 1990, Arthur and Pawliszyn developed the solvent-free sample preparation

method known as solid phase microextraction (SPME). This approach has undergone significant development because of its ease of use, sensitivity, ease of automation, and relatively cheap cost; applications in a variety of fields, including biomedical, environmental, pesticide residue, and medicinal plant analysis, have been documented. Using a modified syringe, this approach extracts the chemicals, which are then mostly desorbed in a gas chromatograph [47]. A unique interface makes it feasible to adapt for LC as well. For SPME analysis, there are two primary extraction methods available: headspace-SPME (HS-SPME), which extracts the chemicals from the sample headspace, and direct SPME, which dips the fiber directly into an aqueous solution. In research, Cannabis oil is subjected to a multi-class pesticide examination employing coated blade spray, solid-phase microextraction, liquid chromatography, and mass spectrometry. 37 pesticides from the initial target list were determined to be appropriate for screening or quantification by CBS, with the latter's performance being verified by LC-MS/MS. The majority of substances were found to attain limits of quantification below or below Health Canada's minimal regulatory limits (majority at 10 ng/mL) while meeting the EU SANTE requirements for analysis (i.e. linearity, precision, accuracy) [48].

Recently for the extraction and analysis of a few pesticides from cannabis samples, a headspace solid-phase microextraction technique and gas chromatography-mass spectrometry were studied. Pesticide analysis was accomplished, and the repeatability of the procedure, measured as the coefficient of variation, varied from 2.4% for bromopropylate to 12.6% for linuron. The single ion monitoring (SIM) limits of detection ranged from 0.014 to 0.83 mg/kg, depending on the pesticide [49].

QuEChERS

Anastassiades and Lehotay initially introduced QuEChERS in 2003 for the detection of pesticide residues in fruits and vegetables. Since then, QuEChERS techniques have grown in acceptance and are now often employed to analyze biological and environmental materials [50]. The Original Buffered approach is the current name for the original QuEChERS technique. The Original Buffered technique was amended by the European Committee for Standardisation (CEN) and the AOAC International organization and published as European Standard EN15662 and AOAC Official method (2007.01), respectively, as official procedures for the detection of pesticides in food samples.

QuEChERS-based approaches offer a wide variety of applications in the investigation of cannabis matrices, but they also have several significant drawbacks. The water content of the test subject should be more than 25% for optimum analyte partitioning in QuEChERS-based techniques. Since cannabis flowers typically contain between 10% and 15% water, each sample needs to be added with water to further hydrate the matrix. It is possible for the matrix effect and interferences to grow during the analysis of samples with dry matrices. Another thing to keep in mind is that combining salts with water, such as $MgSO_4$, in QuEChERS-based techniques might result in heat production via exothermic hydration processes, which could lead to the degradation of heat-labile analytes in the sample combination.

In any case, adding this step to the sample preparation procedure takes time. The salts in the sample extract might deposit in the input liner, which is another problem with GC-based procedures. Additionally, less stable analytes may degrade as a result of pH changes in the sample brought on by the mixing of salts and water. Additionally, a basic adsorbent like primary and secondary amine exchange material (PSA) is frequently utilized as the base sorbent when utilizing dSPE clean-up in QuEChERS procedures since it eliminates various organic acids and sugars that may function as instrument interferences. However, when PSA is utilized, polar pesticide extractions typically result in poorer recoveries.

In recent years, QuEChERS-based technologies have been the most preferred for multi-residue pesticide assessments in cannabis products. To identify 42 pesticides in marijuana, a multi-residue approach based on QuEChERS extraction, followed by liquid chromatography and tandem mass spectrometry, was developed and validated. The sample preparation process is quick and easy when using the original QuEChERS. The relative standard deviations for duplicate injections were less than 4.6%, and coefficients of determination (r2) were more than 0.980 in the range of 1.0 to 50 g kg1. The limits of detection (LOD) and quantification (LOQ) were, respectively, less than 0.32 g kg1 and 1.07 g kg1. Recovery of spiked samples at three different concentration levels (1.0, 5.0, and 50.0 g kg1) in five duplicates was used to confirm precision and accuracy. With an RSD under 6%, recovery values varied from 82 to 119% [51].

To examine the effectiveness and applicability of several technologies for determining the presence of pesticides in marijuana. Through the LC-MS/MS measurement of 61 LC-amenable pesticides, the effectiveness of three common pesticide multi-residue methods—acetate buffered QuEChERS (method A), modified citrate buffered QuEChERS (method B), and citrate buffered QuEChERS (method C)—was evaluated in marijuana. From the 61 target analytes, 37 (method A), 40 (method B), and 46 (method C) chemicals provided accurate findings (70-120% range), taking into account the greatest levels of recoveries for the chosen pesticides in marijuana [52]. Pesticide residues in cannabis-infused candies and beverages may now be found using a quick, accurate, and affordable testing approach. 13 genuine cannabis food and beverage samples were successfully analyzed using the established approach. Bifenazate was found in four out of the 13 samples examined for pesticide analysis, and its presence ranged from 10 to 1221 ng/g [53]. Using QuEChERS sample preparation, LC-MS/MS, and GC-MS/MS, a thorough multi-residue pesticide analysis for cannabis flower matrices was built and validated for 368 pesticides with good recoveries (70-120%) for more than 97% of analytes examined. The approach was used on Oregon cannabis flower samples, and the outcomes were compared to current state pesticide laws. Three of the 100 samples examined had regulated pesticides that were far over Oregon's regulatory action limits, while four of the samples contained pesticides that were not controlled there [54]. Another study reports the analysis of 327 pesticide-active ingredients in cannabis inflorescence using the QuEChERS method combined with gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) and liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS) [55]. Comparative demonstration between the extraction methodologies on the basis of metrits and demerits are given below in table-1.

Table 1: Merits and demerits of sample preparation techniques

S. No	Techniques	Merits	Demerits
1.	Liquid-Liquid extraction	Simple and inexpensive equipment Easy to operate	Minimal selectivity high amount of solvent is necessary, difficult-to-break emulsion
2.	Solid phase extraction	Clean extract, Simple automation, increased compound recovery, applicable to compounds that are soluble in liquid or that are suspended in liquid	High running costs are time-consuming, Direct application to solid matrices is not possible
3.	Solid phase microextraction	ease of use, sensitivity, ease of automation, and relatively cheap cost	Not applicable for solid samples
4.	QuEChERS	Robust result, Highly effective in pesticide residue analysis,	Inapplicable for aqueous samples, multiple steps may Affect the processing

Methods development and validation

Qualitative and quantitative analysis of pesticides in cannabis

The most widely used technique in the field of analytical chemistry since a few decades ago is chromatography. Chromatography is based on the idea that analyte molecules can be applied to a surface or incorporated into a liquid or solid stationary phase, which allows them to be separated while the mobile phase is still in motion. For both qualitative and quantitative analysis, a mixture of analytes is frequently identified, purified, and separated using chromatography methods. Molecular weight, polarity affinity to the mobile and stationary phases, and other variables can affect the separation process.

The chemical composition and polarity of pesticides vary widely. Pesticide analysis methods include gas chromatography (GC) and liquid chromatography (LC) with mass spectrometry (MS). Volatile pesticides should be used GC-MS, whereas ionic and polar pesticides should be used LC-MS.

Gas chromatography-Mass spectrometry

The analytical technique known as gas chromatography-mass spectroscopy (GC-MS) combines the advantages of gas chromatography and mass spectrometry.

The mass/charge ratio is the basis for the analysis in this method. The analytes are conveyed via the inert carrier gas in the gas chromatographic column, where the separation occurs. The interaction of the analytes with the stationary phase provides the basis for the separation. The analyte can be separated from a mixture while the sample is moving through the stationary phase due to differences in physical characteristics, boiling points, and chemical properties. Retention time is defined as the amount of time the analyte takes to elute from the column following injection. The separated analyte exits the column and flows via the controlled temperature transfer line into the MS. Pesticides that are very hydrophobic and volatile are frequently analyzed using GC-MS or GC-MS/MS. Pesticides are resolved on a capillary column using helium as the carrier gas during GC separation. For trace analysis, samples produced from QuEChERS or SPME may be readily fed into the GC inlet in splitless mode and ionized utilizing electron impact (EI) ionization. Quadrupole mass selective analyzer in selected ion monitoring (SIM) mode is used in GC-MS for pesticide identification. One ion is chosen for measurement for each pesticide, and one or two more ions are monitored for identification. To analyze pesticides in cannabis, GC has furthermore been integrated with MS/MS operating on a triple quadrupole mass spectrometer in multiple reaction monitoring (MRM) modes.

In a study to calculate the potential exposure of cannabis users to pesticides and other chemical residues from mainstream cannabis smoke. In the experiment, three different pesticides—bifenthrin, diazinon, and permethrin—as well as the plant growth regulator—paclobutrazol—that are easily accessible to growers in commercial goods were examined. In tandem cooled gas traps, smoke from the smoking devices was condensed before being subjected to gas chromatography-mass spectrometry (GC-MS) analysis [56]. For the study of pesticide residues in cannabis oil, a sensitive and economical sample preparation technique was developed and validated. By applying a planned chosen reaction monitoring approach with a GC-MS/MS and matrix-matched calibration curves, 122 pesticides in the sample were identified. With recoveries ranging from 70% to 120% and within laboratory relative standard deviations below 20%, the technique LOD and LOQ in 3 items were 0.03 and 0.05 mg/kg, respectively [57].

Liquid chromatography-Mass spectrometry

LC-MS is a strong method for analyzing non-polar, and ionic pesticides when compared to GC-MS. Many regulated pesticides are polar and labile, and electrospray ionization (ESI), as well as APCI (Atmospheric pressure chemical ionisation), can be used to quickly ionize them. As a result, LC-ESI-MS/MS and LC-APCI-MS/MS are the best method for analyzing these pesticides as GC/MS and GC-MS/MS finds it difficult to do so. To prevent interference with ESI-MS detection in trace analysis, LC is required to separate pesticides from one another and the intricate cannabis matrix. Reverse phase LC (RPLC) with C8, C18 or biphenyl columns can be used to separate pesticides in these extracts since many pesticides are soluble in an aqueous-organic combination and are extracted using acetonitrile (ACN), ACN/water, Ethyle acetate or Dichloromethane (DCM), then further reconstitute in the suitable solvent like methanol (MeOH) or ACN. During the analysis of non polar pesticide for the threshold ionisation of the analyte modified gradient can be use like 0.1 to 0.5% formic acid or 5 mM to 10 mM of ammonium acetate/ammonium formate in mobile phase. This addition of of ionic availability to provide the excessive electron/charge to the bundle of charged ions and helps to produce the efficient coulambic force for the effective escape of tinny sized ions and reach to the ion guide sampler point.

Consumers and authorities are very concerned about pesticides since many of these substances have unknown health impacts, especially when inhaled. Due to its improved selectivity, sensitivity, and robustness, high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become the method of choice for pesticide detection. This is because it doesn't need considerable sample preparation before analysis. Gas chromatography-tandem

mass spectrometry (GC-MS/MS) techniques have been developed for the identification of pesticides in cannabis samples. These techniques work well but often only analyze fewer analytes than LC-MS/MS. Furthermore, some chemicals, such as abamectin, are not accessible for analysis by GC-MS/MS because they are heat labile and deteriorate in the injection source. GC-MS/MS methods are therefore not as reliable as LC-MS/MS methods, particularly in complex matrices. The absence of regulatory regulations has made it difficult to analyze the pesticides found in cannabis. States have had difficulty creating precise testing regulations, and the testing standards vary as the industry changes. In response to client demands for pesticide analysis, the market has developed several laboratories that provide these services employing LC-MS/MS analyses.

The illegal indoor cannabis farms that feed the Belgian and European cannabis markets cause issues and raise worries regarding the health and safety of intervention workers, firms who dismantle cannabis plants, the immediate surroundings of cannabis plantations, and ultimately cannabis consumers. The main dangers might be caused by pesticide residues on plants, production equipment, and materials; leftover plant growth-promoting compounds; mycotoxins from fungi on harvested plants; and/or excessive quantities of cannabinoids in cannabis plant sections for eating. The chemicals discovered in illegal indoor cannabis crops in Belgium are the subject of the current study. To identify pesticides in indoor cannabis plantations and assess the risks connected to the use, cultivation, and removal of cannabis plants in plantations as well as with dismantling activities in the cultivation rooms, EN15662 QuEChERS extraction method and LC-MS/MS analysis were used. Pesticides were detected in 64.3% of the 72 cannabis plant samples and 65.2% of the 46 samples of carbon filter cloth [58]. Recently a method has been proposed to assess and validate the recently created QuEChERS mega-method for sample preparation to identify pesticide residues in hemp plants, flowers, powders, oils, and pellets using UHPLC-MS/MS. Final extracts for 106 selected pesticides and metabolites from North American monitoring lists were subjected to high-throughput analysis. For more than 80% of the analytes in hemp protein powder, oil, pellets, and fresh plant (dry hemp plant and flower were too complex), multi-level, multi-day validation findings reached 70-120% recoveries with RSDs of 20%. Nearly all pesticides' limits of quantification (LOQs) were 10 ng/g, resulting in 2.8% false negatives out of more than 13,000 analyte values in the spiked samples [59]. According to the EN 15662 procedure, cannabis sample analysis was conducted. UPLC/MS-MS in positive ESI mode with multiple reaction monitoring and GC-MS in scan mode were both used to find pesticides. For a total of 160 distinct pesticides, 50 samples were analyzed. In 19 samples, seven distinct pesticides were found, and five samples had two pesticides. Propamocarb, tebuconazole, propiconazole, and tolylfluanid were four of the chemicals found to be fungicides [13].

Recently sensitivity analysis of 66 pesticides in hemp matrix was performed using an LC-MS/MS system with dual ESI and APCI sources; 62 pesticides were examined using an 18-min LC-MS/MS method with an ESI source, and the remaining 4 pesticides were measured using a 6-min LC-MS/MS method with an APCI source. Results in all 66 pesticides' limits of quantification (LOQ) in hemp were between 0.0025 and 0.1 g/g, which is much less than the state action limits for these analytes in cannabis products in California. For sample preparation, a straightforward, quick, and affordable solvent extraction technique was utilized to achieve an excellent recovery in the region of 80-120% with RSD of less than 20% [60].

Analytical Challenges for Testing Pesticide Residues in Cannabis Samples

Cannabis has an extremely complex matrix composition and comprises components from many different classes, including cannabinoids, terpenes, hydrocarbons, sugars, fatty acids, flavonoids, and others. This makes it difficult to analyze pesticides in cannabis. The fundamental issue with LC-MS/MS is still the sample matrix effect, which causes matrix interference and variable signal ion suppression [36]. Due to the wide variation in the high concentration levels of naturally occurring cannabinoids and the high terpene content, it is also challenging to quantify pesticide residues in cannabis.

Since several nonpolar and chlorinated pesticides are difficult to ionize with an electrospray ion source, the study of pesticides in cannabis and other food matrices is typically conducted using both GC-MS/MS and LC-MS/MS [61, 62].

Usually, the dirty matrix seen in cannabis samples would soon foul the traditional GC-MS/MS and LC-MS/MS systems, increasing the maintenance costs and downtime and leading to a loss of production [63]. We demonstrated that the LC-MS/MS technique created in this study would be more resistant to contamination by the soiled cannabis matrix. Because it is not feasible to transmit the eluate directly from the LC column to the MS source, the mass spectrometer equipment is not completely compatible with the LC system. To effectively transport the separated components from the LC column to the MS source, the LC-MS/MS system has an interface. The MS system's ionization effectiveness and vacuum conditions shouldn't be hampered by the interface. Atmospheric pressure photo-ionization (APPI), atmospheric pressure chemical ionization (APCI), and electrospray ionization (ESI) are now the most extensively used LCMS/MS interfaces. Comperative parameters conformatory quantification mass spectroscopy methods are given below in Table-2.

Table 2: Merits and demerits of Gas and Liquid Chromatography mass spectrometry

S. No	Techniques	Merits	Demerits
1.	Gas Chromatography-Mass spectrometry	High separation efficiency, easily separates the isomers, robust results.	The compound must be volatile.
2.	Liquid Chromatography	High reproducibility and repeatability, lower detection limit, robust results, long range of analyte identification	Typically identify isomers, difficulty in the analysis of organochlorine pesticides and polar pesticides.

In recent years many of the studies showed the monitoring and analysis of the various produces of the cannabis and its raw plant which gives us the brief availability to monitor and establish a regulatory advosry related to the contaminants in cannabis produces. The summarized details of the several studies with analytical techniques used for the quantification given below in the Table-3.

Table 3: Recent study for pesticide residue analysis in cannabis and its products

S. No	Analytes	Matrix	LOQ	Sample preparation technique	Analytical instrument	Mode of ionization and column used	References
1.	42 pesticides	Marijuana	1.07 µg/kg	QuEChERS	LC-MS/MS	ESI	[51]
2.	74 Pesticides	Cannabis oil	2.5-25 ng/g	SPME	LC-MSMS	ESI	[48]
3.	39 pesticides in cannabis leaves and 40 pesticides in cannabis oil, 32 pesticides in cannabis flowers	Cannabis leaves, flower and oil	0.01 µg/g and 0.02 µg/g	SPE and QuEChERS	HPLC-MS/MS And GC-MS/MS	ESI and EI	[12]
4.	66 Pesticides	Cannabis oil	-	LLE	LC-MS/MS	ESI and APCI	[41]
5.	61 pesticides	Marijuana	10-200 ng g^{-1}	QuEChERS	LC-MS/MS	ESI	[52]
6.	82 pesticides	Cannabis products	0.02–5 µg/L	LLE	LC-MS/MS	ESI	[64]
7.	40 pesticides	Cannabis leaves and oil	0.01 µg/g 0.02 µg/g	LLE and SPE	LC-MS/MS and GC-MS/MS	EI And ESI	[20]
8.	122 pesticides	Cannabis oil	0.03-0.05 mg/kg	QuEChERS	GC-MS/MS	EI	[57]
9.	93 pesticides	Cannabis leaves, oil, pellets	< 10 ng/g	QuEChERS	UHPLC-MS/MS	ESI	[59]
10.	35 pesticides	Cannabis Edibles and Beverages	-	QuEChERS	LC-MS/MS	ESI	[53]
11.	160 pesticides	Cannabis plant	5 ng/g	QuEChERS	LC-MS/MS And GC-MS/MS	ESI and EI	[13]
12.	12 pesticides	Cannabis samples	0.014 and 0.83 mg/kg,	SPME	GC-MS/MS	EI	[49]
13.	327 pesticides	Cannabis Inflorescence	0.01 µg/g	QuECHERS	GC-MS/MS And LC-MS/MS	EI and ESI	[55]
14.	200 Pesticides	Cannabis leaves	20-500 ng/g	QuECHERS	LC-MS/MS	ESI	[65]

Future scope and conclusion

As interest in using cannabis for food, cosmetics, or medical purposes grows, analysis of pesticides in cannabis and associated products has been studied. To remove pesticides from cannabis and associated products, QuEChERS and SPE techniques were most frequently used. Then, considering that many families of pesticides or pollutants may be concurrently separated, chromatographic methods (GC and LC) have been frequently utilized in multi-residue analysis, expanding the breadth of the studies. Since LRMS offers the capacity for quantification, it has been widely employed in connection with detection methods up to this point, being QqQ the analyzer typically used in regular laboratories.

Thus, it is possible to identify hundreds of pesticides in a single run, but only focused analysis is possible. Since HRMS offers appropriate performance for quantitation as well as higher adaptability (targeted and non-targeted analysis), it is being employed more and more. Given that they each have distinct advantages, HRMS and LRMS may coexist shortly. To analyze all of the components in the sample and enable retroactive examination of samples, generic analytical procedures for hundreds of chemicals can be applied. Moreover, the sample can be reanalyzed later to look for pollutants and other harmful substances that weren't of concern at the time.

Conclusion

In conclusion, to satisfy legal requirements, LC-MS/MS and GC-MS/MS can offer complimentary analyses of pesticides in cannabis. The whole list of pesticides from California and Oregon's lists can be analyzed using LC-MS/MS, however, this needs sophisticated MS apparatus, such as a dual ionization source and a complex analytical approach. GC is a considerably more convenient approach with excellent sensitivity and selectivity for several pesticides, including chlorinated pesticides and herbicides. The advantages of using both GC-MS and LC-MS methods for the study of pesticides have been highlighted in both peer-reviewed literature and technical notes.

Acknowledgments

The authors would like to thank the Department of Chemistry, K.S. Saket P G College, Ayodhya and Dr. Ram Manohar Lohia Avadh University, Ayodhya, Uttar Pradesh 224001 for its support and infrastructure to ensure the success of this study.

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