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Global Journal of Medical, Pharmaceutical, and Biomedical Update

Mini-Review

Application of HPLC in Biomedical Research for Pesticide and Drug Analysis

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Received: 14 May 2023
Accepted: 02 July 2023
Published: 07 September 2023

DOI
10.25259/GJMPBU_40_2023

Quick Response Code:

ABSTRACT

Compared to traditional liquid chromatography, high-performance liquid chromatography (HPLC) delivers better results for analyzing unknown compounds. It permits faster resolution time, better peak shapes, repeatable responses, and greater precision. A comprehensive literature search has been conducted using online academic databases such as Google Scholar, PubMed, Web of Science, and Scopus, using keywords such as HPLC, pesticide analysis, drugs analysis, chromatographic conditions, and HPLC Column type. Total 75 number of articles were collected from peer-reviewed journals. With the help of literature review we have summarized the chromatographic condition of 30 drug candidates and 27 pesticide candidates. The study's findings can guide future researchers to understand the chromatographic parameters of drugs and pesticides.

Keywords: High-performance liquid chromatography, Pesticides analysis, Drug analysis, Chromatographic conditions, Column type

INTRODUCTION

Separation techniques play a significant role in analysis, and chromatography is a robust separation method utilized in all research fields.[1] Chromatography passes a solution through a column filled with a suitable adsorbent, where the solutes are deposited in bands on the surface of a material. The bands move at different speeds when a pure solvent is introduced through the column.[2] Molecular properties linked to adsorption, partition, affinity, or discrepancies between their molecular weights are among the elements that impact this separation process. These variations lead specific mixture components to spend more time in the stationary phase and travel more slowly through the chromatographic system.[3] The word "chromatography" was first used in 1903 by the Russian botanist Mikhail Tswett. He employed liquid column chromatography, in which the mobile phase was a liquid and the stationary phase was a solid adsorbent loaded into a glass column. Using nearly 100 adsorbents, he studied chlorophyll extracts in petroleum spirit.[4-6] James and Martin documented the first analytical application of chromatography in 1952 when they used gas chromatography (GC) to analyze fatty acid compounds. There are several different forms of chromatography. Size, binding, affinities, charge, and other parameters are used in various chromatographic methods. Column chromatography, high-performance liquid chromatography (HPLC), GC, size exclusion chromatography, ion exchange chromatography are examples of different chromatography.[7]

HPLC is a type of liquid chromatography in which separation (or partition) happens between a mobile phase (the solvent) and a stationary phase (the column packing).[8] HPLC is widely utilized in qualitative and quantitative examination of many types of compounds. Validating a method is a crucial step in HPLC analysis. Determining whether an analytical technique is appropriate for the function is known as "analytical method validation." Cost, simplicity, operator expertise, availability, and other factors are secondary to the actual validity of the approach under consideration when selecting an analytical method. During the validation phase, the following attributes are frequently tested: Specificity, robustness, linearity, precision, accuracy, limit of detection, limit of quantification, and solution stability.[9] HPLC is frequently used in the study of steroids since it offers an excellent tool for separation and quantification.[10] Reversed-phase mode separation is the preferred HPLC technique for all chemical classes. In reversed phase-HPLC, octadecyl silica (ODS or C18) columns are frequently employed as the stationary phase. Other materials can also be used to give various selectivity levels, including C8, C2, phenyl, amino, and cyano phases.[11] The chemistry of the mobile phase also affects selectivity. The mobile phase, which may be utilized in the isocratic or gradient mode, is typically prepared by mixing methanol or acetonitrile with varying amounts of water.[12] This paper summarizes the fundamental analytical criteria of HPLC such as column type, column temperature, mobile phase composition, flow rate, and detector type for 27 pesticides and 30 drug candidates.

APPLICATION OF HPLC IN PESTICIDES ANALYSIS

Pesticides, or antiparasitic chemicals used in agriculture, have quickly expanded in the past 30 years due to the advancement of organic synthetic chemistry.[13] Nowadays, over a hundred different pesticides are commonly used to protect plants. The problem of food contaminated with pesticides is a source of worry for practically everyone and everywhere. Several developed nations have implemented frequent monitoring programs for pesticide exposure control. These programs measure the extent of contamination in food items and highlight probable instances when pesticide residues surpass their tolerance thresholds due to poor farming practices.[14] Pesticide residues beyond the acceptable boundaries in vegetables during harvest are a significant cause for concern worldwide and nationwide.[15] The improper, wasteful, and unethical application of pesticides exacerbates the severity of the residue problem. Food products are dangerous for human consumption and export due to these residues. Furthermore, the residues harm the ecosystem.[16] As a result, applying more susceptible and selective analytical techniques to monitor pesticide residue quantities and regulate the biomagnification process is necessary due to the correspondingly enhanced intake of agrochemical pollutants into the environment. Much progress has been achieved in creating and utilizing various analytical techniques, including separation techniques such as GC and HPLC and detection methods such as electrochemistry, spectrophotometry, and spectrofluorimetry.[17] HPLC is increasingly used, particularly for the study of pesticides that GC cannot determine directly due to the compounds' weak volatility, polarity, or thermal stability.[18] High-quality liquid chromatography with diode-array detection (DAD) can accurately identify pesticides in complex mixtures. These techniques have enabled detecting and quantifying pesticide residues in various atmospheres and food substances.[19-21] Creating a susceptible and highly accurate method is essential for accurately determining and measuring the analytes in complex matrices (such as food products). European Union directives specify the maximum residue levels for pesticides allowed in goods of plant or animal sources suitable for consumption by humans or animals.[22] With many pesticides in each analysis (injection), developing multi-residue technologies for pesticide analysis is crucial.[22] A comprehensive literature survey revealed that many solvents, including acetone or ethyl acetate, petroleum ether, n-hexane, and methylene chloride, have been employed to extract pesticide residue from fruits and vegetables. In this mini-review, we have summarization of 27 pesticide candidates [Table 1] with their chromatographic condition. The chromatographic information of the 27 pesticide candidates was collected from PubMed, NCBI, Google Scholar, Scopus, Web of Science databases.[23-42]

APPLICATION OF HPLC IN DRUG ANALYSIS

HPLC is a significant analytical technology used throughout the whole drug development, formulation, and manufacturing process in the newer pharmaceutical sector.[43] The use of liquid chromatography techniques in pharmaceutical analysis presents a potent weapon for clinical studies as well as pharmacological medication evaluation. Compared to earlier LC procedures, HPLC techniques have several benefits. They permit faster resolution time, better peak shapes, repeatable responses, and greater precision. HPLC columns do not need to be repackaged before use. Higher pressures can also be introduced to the solvent flow using HPLC columns.[44,45]

Table 1: Summarization of pesticides with their chromatographic condition.

Pesticides	Matrix	Column	Column temperature	Mobile phase	Flow rate	Detector (nm)	Ref.
2,4-Dichlorophenoxyacetic acid (2,4-D)	Rat serum	C_{18}	40°C	A=Acetonitrile B=0.02 M ammonium acetate (containing 0.1% formic acid)	1.0 mL/min	UV 230	[23]
3-Hydroxy carbofuran	Coconut water	C_{18}	Room temperature	A=Acetonitrile B=Water	1.0 mL/min	UV 275	[24]
Carbofuran	Coconut water	C_{18}	Room temperature	A=Acetonitrile B=Water	1.0 mL/min	UV 275	[24]
Acetamiprid	Postmortem human blood, liver, stomach	RP 80	40°C	Acetonitrile: Water (50:50 v/v)	1.0 mL/min	UV 248	[25]
Alachlor	Soils	C_{18}	60°C	25 mM dipotassium hydrogen phosphate pH – 7.0: ACN (80: 20 v/v)	1.0 mL/min	UV 210	[26]
Metolachlor	Soils	C_{18}	60°C	25 mM dipotassium hydrogen phosphate pH – 7.0: ACN (80: 20 v/v)	1.0 mL/min	UV 210	[26]
Aldicarb	Vegetables and fruits	C_{18}	40°C	A=Water B=Acetonitrile	1.2 mL/min	UV 210	[27]
Aldicarb sulfone	Vegetables and fruits	C_{18}	40°C	A=Water B=Acetonitrile	1.2 mL/min	UV 210	[27]
Aldicarb sulfoxide	Vegetables and fruits	C_{18}	40°C	A=Water B=Acetonitrile	1.2 mL/min	UV 210	[27]
Benfuracarb	Soil and water	ODS	Room temperature	A mixture of acetonitrile-water (13: 7)	1.0 mL/min	UV 280	[28]
Benomyl	Apple foliage	ODS	Room temperature	ACN: H_2O: Buffer (23:72:5% v/v) pH-7	0.8–1.5 mL/min	UV 280	[29]
Carbendazim	Apple foliage	ODS	Room temperature	ACN: H_2O: Buffer (23:72:5% v/v) pH-7	0.8–1.5 mL/min	UV 280	[29]
Buprofezin	Urine, serum, tomato, soil	C_{18}	25.0°C	Acetonitrile: Buffer 75:25 (v/v)	1.0 mL/min	UV 254	[30]
Carbosulfan	Oranges	ODS	42°C	Acetonitrile: Water 75:25 (v/v)	1.0 mL/min	Fluorescence detector 330/465	[31]
Diazinon	Water and soil	C_{18}	Ambient temperature	Acetonitrile: Water 65:35 (v/v)	1.0 mL/min	UV 245	[32]
Fenitrothion	Water and soil	C_{18}	Ambient temperature	Acetonitrile: Water 65:35 (v/v)	1.0 mL/min	UV 245	[32]
Dithianon	Red pepper	C_{18}	35°C	1% AcOH in MeOH-H_2O (60:40, v/v)	1.0 mL/min	UV 263	[33]
Fenarimol	Blood, liver, and kidney samples	C_{18}	30°C	Acetonitrile: Water 60:40 (v/v)	0.250 mL/min	UV 225	[34]

(Contd...)

Table 1: *(Continued)*

Pesticides	Matrix	Column	Column temperature	Mobile phase	Flow rate	Detector (nm)	Ref.
Hexaconazole	Pesticide formulation	C_{18}	30°C	A=ACN+MeOH (80+20) B=Water (0.1% TFA) 60:40 (v/v)	1.0 mL/min	PDA detector 205	[35]
Imidacloprid	Water and soil	ODS	25°C	Acetonitrile: Water 20:80 (v/v)	1.5 mL/min	UV 270	[36]
Lufenuron	Napa cabbage	C_{18}	Room temperature	Methanol: Water 75:25 (v/v)	1.0 mL/min	UV 220	[37]
Chlorfenapyr	Napa cabbage	C_{18}	Room temperature	Methanol: water 75:25 (v/v)	1.0 mL/min	UV 220	[37]
Metalaxyl-M	Soil and sunflower plants	Chiralcel OJ column	Room temperature	n-hexane: 2 propanol (15%v/v)	0.8 mL/min	UV 254	[38]
Oxadiazon	Pesticide formulation	C_{18}	Room temperature	Acetonitrile: Water 80:20 (v/v)	1.0 mL/min	UV 292	[39]
Pendimethalin	Soil and garlic	C8	Room temperature	Acetonitrile: Water 80:20 (v/v)	1.0 mL/min	UV 240	[40]
Pyrazosulfuron-Ethyl	Soils	C_{18}	30°C	MeOH - H_2O (0.2% Formic acid) 75:25 (v/v)	1.0 mL/min	UV 241	[41]
Sulfosulfuron	Soils and wheat grain	RP-8	Room temperature	Acetonitrile: Water 80:20 (v/v) Or ACN: H_2O: H_3PO_4 80:20:0.1 (v/v/v)	1.0 mL/min	UV 212	[42]

**AcOH: Acetic acid, ACN: Acetonitrile, MeOH: Methanol, TFA: Trifluoroacetic acid, H_2O: Water, H_3PO_4: Phosphoric acid

Table 2: Summarization of drug with their chromatographic condition.

Drugs	Column	Column temperature	Mobile phase	Flow rate	Detector (nm)	Ref.
Amoxicillin	C_{18}	Ambient	Buffer: ACN (90:10% v/v) pH-7	1.0 mL/min	UV 254	[47]
Aprepitant	C_{18}	Ambient temperature	Methanol: Water (90:10% v/v)	1.0 mL/min	UV 220	[48]
Cinitapride	C_{18}	Room temperature	0.1% HCOOH in H_2O: ACN	0.5 mL/min	UV 268	[49]
Dexrabeprazole	C_{18}	Room temperature	ACN: 0.025M KH_2PO4 30:70 (v/v)	1.0 mL/min	UV 284	[50]
Dimenhydrinate	C8	Room temperature	0.05M KH_2PO4: Methanol (35:65, v/v)	1.0 mL/min	DAD 240	[51]
Diphenhydramine	C_{18}	Room temperature	MeoH: ACN: H_2O: 10mM Heptane sulfonate and 13 mM Triethylamine, (10:26:64)	1.0 mL/min	UV 254	[52]
Domperidone	C_{18}	Room temperature	MeoH : KH_2PO4 (65:35% v/v) pH-3	1.0 mL/min	UV 227	[53]
Esomeprazole	C_{18}	Room temperature	ACN: Phosphate buffer (50:50% v/v)	1.0 mL/min	UV 302	[54]
Hydrocortisone	C_{18}	Room temperature	MeoH: H_2O: Acetic acid (60: 30: 10, v/v/v)	1.0 mL/min	UV 254	[55]
Hyoscine	C_{18}	30°C	A=0.01M K2HPO4 containing 2 g/L heptane sulfonic acid sodium salt, pH-3 B=Acetonitrile, 80% v/v	2.0 mL/min	DAD 210	[56]

(Contd...)

Table 2: *(Continued)*

Drugs	Column	Column temperature	Mobile phase	Flow rate	Detector (nm)	Ref.
Ilaprazole	C_{18}	Room temperature	Methanol: Water (70:30% v/v) pH-3.0	1.0 mL/min	UV 237	[57]
Itopride	C_{18}	Room temperature	A=Buffer 1.4 mL *ortho*-phosphoric acid at pH-3.0 with triethylamine B=Acetonitrile	1.0 mL/min	UV 220	[58]
Lafutidine	C_{18}	Room temperature	0.02M K_2HPO_4: ACN (30:70, v/v)	1.0 mL/min	UV 215	[59]
Meclozine hydrochloride	C8	Room temperature	0.2% triethylamine in water: Methanol (65:35, v/v)	1.0 mL/min	PDA 229	[60]
Mosapride	C_{18}	40°C	Methanol: 0.02M K2HPO4 (70:30, v/v)	1.1 mL/min	UV 274	[61]
Omeprazole	C_{18}	40±1°C	Phosphate buffer (pH 7.4): ACN (70:30 v/v)	1.5 mL/min	UV 280	[62]
Prucalopride	C_{18}	Room temperature	0.1% H_3PO_4: MeoH (30:70 v/v)	1.0 mL/min	UV 225	[63]
Rabeprazole	C_{18}	Room temperature	MeoH: H_2O (65:35 v/v)	0.8 mL/min	UV 284	[64]
Donepezil	C8	50°C	Buffer: Methanol: Triethylamine (55:45:5 v/v)	1.0 mL/min	PDA 271	[65]
Flavoxate	C8	35°C	ACN: MeOH: 0.1% HCOOH (5:20:75%v/v)	1.0 mL/min	UV 311	[66]
Homatropine	C8	Room temperature	ACN: Potassium dibasic phosphate 10 m Mol/L PH--6.9 (35:65 v/v)	1.0 mL/min	UV210	[67]
Pilocarpine	C_{18}	25°C	A=Phosphoric acid at pH-3.0 with triethylamine B=MeOH (90:10 v/v)	1.0 mL/min	DAD 215	[56]
Carbimazole	C_{18}	Room temperature	MeoH: 0.1% H_3PO_4 (80:20 v/v)	0.7 mL/min	UV 291	[68]
Hydrocortisone	RP-column	40°C	ACN: Buffer (75:25% v/v)	1.0 mL/min	UV 254	[69]
Pioglitazone	C8	Room temperature	ACN: 140mM KH_2PO4 (40:60% v/v)	1.4 mL/min	UV 269	[70]
Azathioprine	C_{18}	Room temperature	ACN: H_2O (50:50% v/v) pH-3.3	1.0 mL/min	UV 276	[71]
Cytarabine	C_{18}	Room temperature	ACN: Buffer (Ammonium acetate) (30:70% v/v)	1.0 mL/min	UV 272	[72]
Melphalan	C_{18}	Ambient	ACN: H_2O: 1% H_3PO_4 (70:27:03%v/v)	1.0 mL/min	UV 275	[73]
Oxaliplatin	C_{18}	25±2°C	0.01 M phosphoric acid: Acetonitrile (95:05% v/v)	1.0 mL/min	UV 255	[74]
Vincristine	C_{18}	Ambient	0.02 M phosphate buffer, pH-5.4: Acetonitrile (50:50% v/v)	1.0 mL/min	UV 233	[75]

**KH_2PO4: Potassium dihydrogen orthophosphate, H_3PO_4: Phosphoric acid, AcOH: Acetic acid, ACN: Acetonitrile, MeOH: Methanol, TFA: Trifluoroacetic acid, H_2O: Water, DAD: Diode-array detection, K2HPO4: Dipotassium phosphate

In the past 20 years, the rapid advancement of HPLC has allowed scientists to identify and quantify organic molecules, including pharmaceuticals and medication ingredients.[46] Scientists worked hard to discover a new method to fast-track their research. The drug industry tries to decrease research and innovation time and expenditures. For the development of chromatographic conditions, scientists tried to achieve their goal.[45] In this mini-review, we have summarization of 30 drug candidates [Table 2] with their chromatographic condition. The chromatographic information of the 30 drug candidates was collected from PubMed, NCBI, Google Scholar, Scopus, Web of Science databases.[47-75]

CONCLUSION

HPLC is a popular method for the analysis of pesticides and drugs. Determining pesticides is crucial because even minute amounts of a compound can be hazardous or detrimental to health. In drug analysis, HPLC is employed to find out pure compounds quickly. This review article helps researchers to know the chromatographic condition required for analyzing some common pesticides and drug molecules.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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How to cite this article: Saha S, Mallik S, Debnath B, Singh WS, Ashif Ikbal A, Manna K. Application of HPLC in biomedical research for pesticide and drug analysis. Glob J Med Pharm Biomed Update 2023;18:20.