



Understanding applications of chromatographic techniques in fisheries: present status and future perspective

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Abstract

Gas Chromatography (GC), Gas Chromatography-Mass Spectrometry (GC–MS), and High-Performance Liquid Chromatography (HPLC) represent fundamental analytical techniques in fisheries research, particularly for the detection, separation, and quantification of various contaminants and biochemical compounds. GC is primarily employed for the analysis of volatile organic compounds, while the coupling of GC with mass spectrometry (GC–MS) enhances specificity and sensitivity, allowing for the precise identification of trace organic pollutants. In contrast, HPLC is better suited for the analysis of non-volatile, polar, or thermally unstable compounds, such as antibiotics, proteins, and hormones. This review conducts a comprehensive comparative analysis of the applications of GC, GC–MS, and HPLC in fisheries, with a focus on their current utilization in environmental monitoring, food safety, and fish toxicology. Furthermore, it evaluates their limitations and offers insights into future advancements, including automation, miniaturization, and artificial intelligence-driven data analysis. By exploring these developments, this review provides a robust framework for selecting the most appropriate chromatographic method for specific research applications, ultimately underscoring the significance of these technologies in advancing sustainable fisheries management and environmental protection.

Keywords Chromatography · Detectors · Application · Fisheries

Introduction

Gas Chromatography (GC), Gas Chromatography-Mass Spectrometry (GC–MS), and High-Performance Liquid Chromatography (HPLC) are widely recognized as essential tools in analytical chemistry, especially for separating, identifying, and quantifying complex mixtures of chemical compounds (Ni et al. 2012). Each technique has unique strengths that make it suitable for specific types of analyses. GC is particularly effective for separating volatile organic compounds by vaporizing a sample and passing it through a column, where components are separated based on their

boiling points and interactions with the stationary phase (Lehotay and Hajslova 2002). GC–MS takes this process a step further by coupling GC with mass spectrometry, allowing for the precise identification of organic compounds even at trace levels (Hernandez et al. 2011). In contrast, HPLC is ideal for analyzing non-volatile, polar, or thermally unstable compounds, using high pressure to push liquid samples through a column, facilitating separation based on polarity and molecular size (Kataoka 2023).

In fisheries, these analytical techniques have become indispensable for monitoring the chemical environment and assessing the health of aquatic species (Connon et al. 2012). Their applications range from detecting pollutants such as pesticides, hydrocarbons, and heavy metals in fish tissues and water bodies to identify biomarkers of environmental stress or toxic exposure (Vander oost et al. 2003). These techniques also play a crucial role in ensuring (Sheng and Wang 2021). The ability to detect both organic and inorganic compounds at trace levels has positioned GC, GC–MS, and HPLC as critical for regulatory compliance and environmental monitoring in fisheries science.

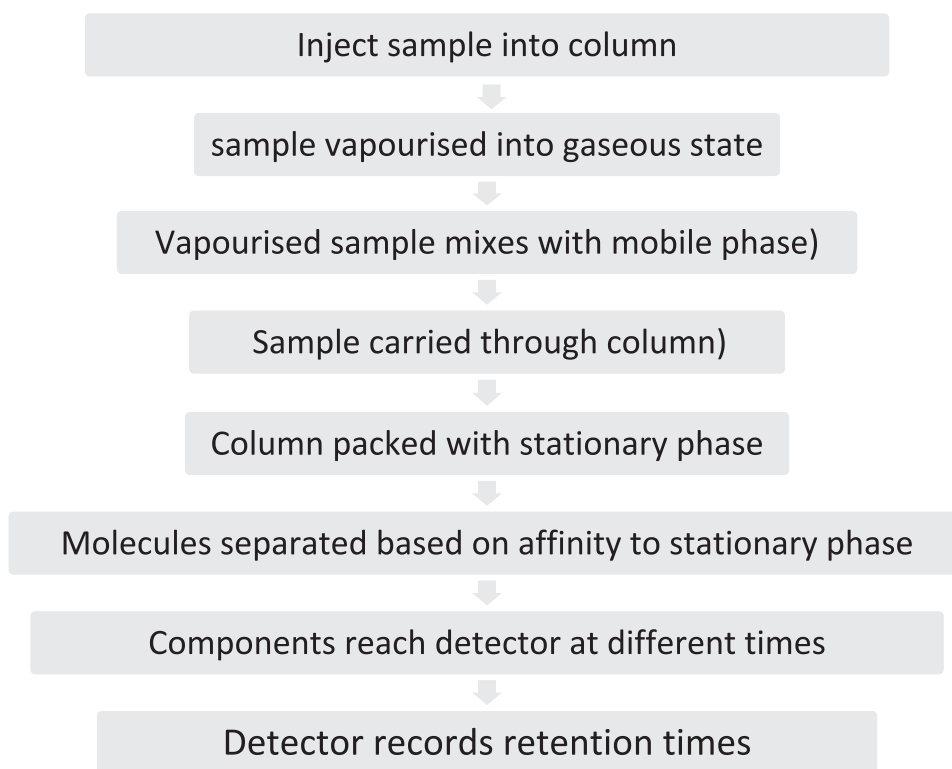
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Flowchart 1 Gas chromatography procedure



This review aims to provide a comparative analysis of the applications of GC, GC–MS, and HPLC in fisheries, examining their current status and potential future developments. By exploring their strengths and limitations, this review seeks to offer researchers valuable insights into selecting the most appropriate technique for their specific needs. Additionally, it highlights emerging trends and advancements that could further enhance the effectiveness of these analytical methods in fisheries research.

The application of GC, GC–MS, and HPLC in fisheries research offers a robust means of analyzing various contaminants and biomolecules in aquatic organisms. Each technique presents distinct principles and capabilities, making them well-suited for specific types of compounds.

GC and its relevance in fisheries

Principle

Gas Chromatography (GC) separates molecules based on their retention time, which is determined by their interaction with the stationary phase. When injected, the sample—whether liquid or gas—is vaporized and transported by a mobile phase, typically helium, through the column (Bartle and Myers 2002). Molecules with higher affinity for the stationary phase exhibit longer retention times, while those with less affinity elute faster (Grob and Barry 2004;

Littlewood 2013). The process as shown in Flowchart 1 involves injecting the sample into the column, where it vaporizes, mixes with the mobile phase, and travels through the stationary phase, separating components based on their affinities. These components are then detected and collected as they exit the column at different times [Fig. 1] (Jennings et al. 1997).

In fisheries research, GC detectors are instrumental in monitoring contaminants, assessing fish metabolism, and analyzing environmental toxins, such as pesticides and hydrocarbons that accumulate in aquatic organisms (Kaur and Sharma 2018).

The choice of detector can significantly impact the accuracy of the results, depending on the target analytes and their concentration levels. Detectors such as flame ionization detectors (FID), thermal conductivity detectors (TCD), and electron capture detectors (ECD) are commonly used, each with its own advantages based on the nature of the compounds being analyzed. Table 1 provides a summary of the different types of detectors used in GC, detailing their description, sensitivity levels, and the specific analytes they are commonly employed to detect in fishery-related studies.

Gas chromatography-mass spectrometry (GC–MS)

GC–MS analyzes liquid, gaseous, or solid samples by first vaporizing and separating them via gas chromatography (Mastovska and Lehotay 2003). Compounds are propelled



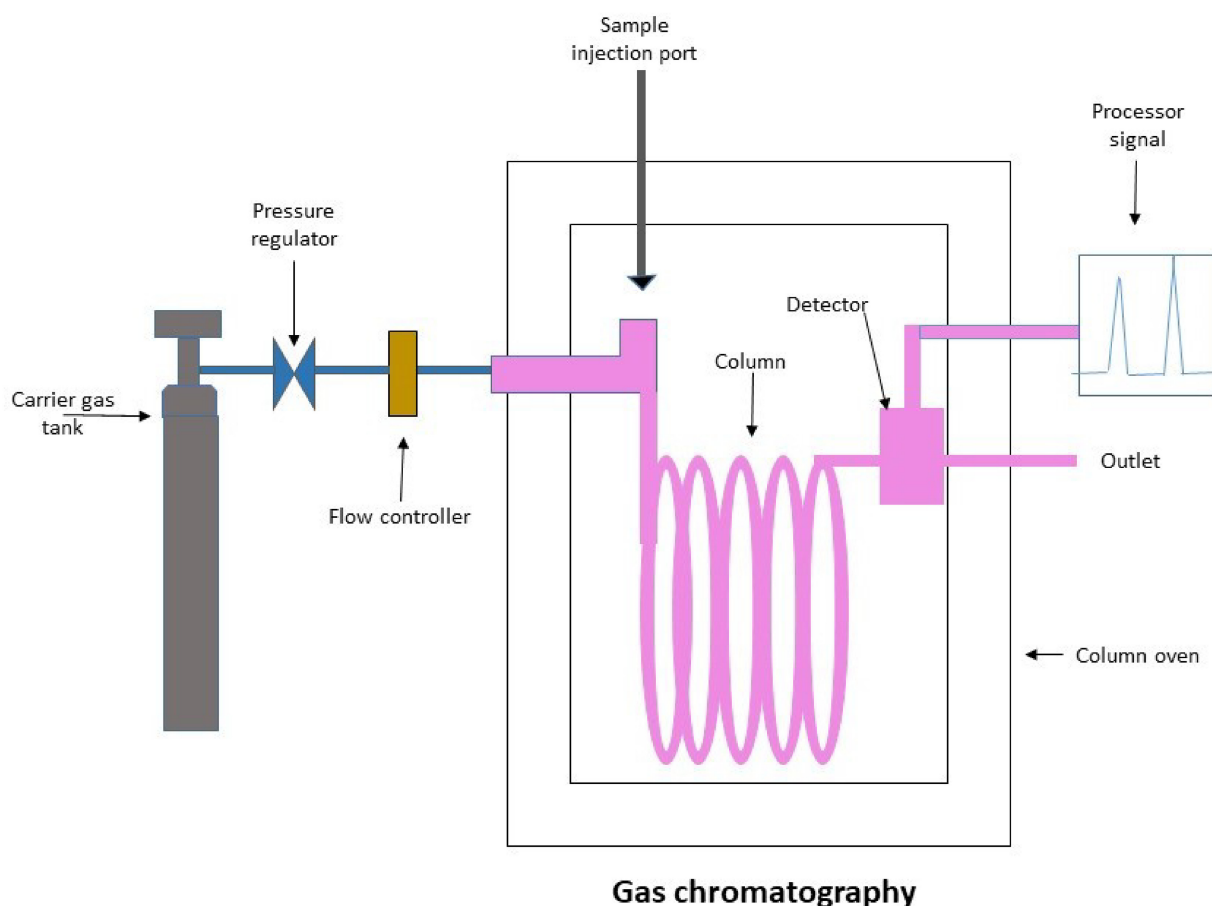


Fig. 1 Schematic of GC workflow (Original design)

through a capillary column by an inert carrier gas, such as helium or nitrogen, and are separated based on their retention times, which depend on boiling points and polarity. The mass spectrometer then ionizes and fragments the separated compounds using electron or chemical ionization. A quadrupole or ion trap mass analyzer sorts the ions by their mass-to-charge (m/z) ratios. Data can be acquired in full scan mode for a wide m/z range or in selected ion monitoring (SIM) mode for targeted analysis (Santos and Galceran 2003). Table 2 presents a comprehensive overview of the principles, key components, detectors, applications, and significant findings of gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), and high-performance liquid chromatography (HPLC) in fisheries research. This table highlights the fundamental working principles of these chromatographic techniques, their essential components, the types of detectors commonly used, and their wide-ranging applications in analyzing various fish-derived compounds.

In ion detection, fragmented ions are identified by their mass-to-charge (m/z) ratios, with peak areas indicating compound abundance. Gas chromatography-mass

spectrometry (GC–MS) generates multiple chromatographic peaks, each linked to a distinct mass spectrum for compound identification (Sparkman et al. 2011). Commercial mass spectral libraries aid in identifying and quantifying unknown compounds. For example, trace aniline and its derivatives were detected in environmental samples using capillary GC–MS after liquid–liquid extraction and steam distillation (Falih dahia 2018; Christie 1998; Raina 2011).

Gas chromatography-tandem mass spectrometry (GC–MS/MS), employing a triple quadrupole system, offers increased selectivity and sensitivity, particularly in pesticide analysis and environmental contaminant detection. Operating in selective reaction monitoring (SRM) mode, GC–MS/MS minimizes background ion interference, enhancing detection capabilities (Krone et al. 2010; Tsikas and Zoerner 2014). Figure 2 highlights the GC–MS workflow.

Table 1 Applications of gas chromatography detectors in fisheries science

Detectors	Description	Analytes	Application in fisheries science	References
Flame Ionization Detector	Employed as a versatile GC detector. Upon sample elution from the column, it undergoes combustion in a hydrogen/air flame, and the resulting ionized particles are quantified as an electric current	Most organic compounds	Detection of hydrocarbons, fatty acids, and other organic compounds in fish tissues and aquatic environments	Kananen et al. (2000)
Thermal conductivity detector	Capable of detecting any sample component with a distinct thermal conductivity compared to the carrier gas. It utilizes a solitary filament powered by a constant current DC supply. Operationally, it solely relies on one gas type, typically chosen as the carrier gas, which may include helium, argon, nitrogen, or hydrogen	Low-molecular-weight hydrocarbons, permanent gases, fatty acids, as well as aromas and scents	Monitoring of dissolved gases and volatile organic compounds in water samples from fisheries	Karim et al. (2012)
Nitrogen-phosphorus detector	A nitrogen phosphorus detector is customarily engineered to discern the two elements mentioned in its name, utilizing the modifications they induce in the behavior of a metal bead	Organophosphates (phosphorus containing pesticides) and drugs (Nitrogen containing compounds)	Detection of organophosphate and nitrogen-based pesticides in fish tissues and aquatic environments	Boltes and Gonzalez (2013)
Electron capture detector	Electron capture detectors identify gas molecules by capturing electrons and binding them to analyte molecules, subsequently gauging the resultant current alteration in a detection chamber, directly correlating with analyte concentration	Halogenated organic compounds, aromatic compounds, other analytes with high electron affinity (e.g., organometallics, nitriles, or nitro compounds)	Detection of trace levels of chlorinated pesticides and PCBs in fish tissues and water samples	Rahman et al. (2021)
Flame photometric detector	The flame photometric detector entails the combustion of compounds within a flame, followed by the utilization of a photomultiplier tube to identify spectral lines emitted by specific elements	Sulphur and phosphorus containing organic compounds i.e., pesticides and petroleum streams	Analysis of sulphur and phosphorus-containing pesticides and other contaminants in fish tissues	Campone et al. (2010)
Sulphur Chemiluminescence Detector	Based on light-generating reaction between ozone and sulfur monoxide, which originates from the combustion of analytes present in the sample	Sulphur containing compounds like fuels, fragrances, food flavours, beverages etc	Detection of sulfur-containing contaminants and compounds in fish tissues and aquatic environments	Hijazi (2017)
Nitrogen Chemiluminescence Detector	Precisely engineered to discern the two elements mentioned in its name, contingent upon their influence on the functionality of a metallic bead	Compounds containing nitrogen (e.g., substances found in chemicals, environmental contaminants, food and beverages, fuels, gases, pesticides and herbicides, petrochemicals, polymers, as well as nitrosamines in pharmaceuticals)	Analysis of nitrogen-containing contaminants in fish and water samples	Scott (2003)

Table 1 (continued)

Detectors	Description	Analytes	Application in fisheries science	References
Mass Spectrometry (MS)	Ionizes and fragments compounds; sorts ions by mass-to-charge ratio (m/z)	Organic pollutants, volatile organic compounds	Trace-level detection of pollutants, steroid hormones, and lipids	Santos and Galceran (2003)
Tandem Mass Spectrometry (MS/MS)	Uses multiple stages of mass spectrometry for enhanced selectivity and sensitivity	Pesticides, environmental contaminants	High-sensitivity detection of pesticide residues in aquatic samples	Krone et al. (2010)

Applications of GC and GC–MS in fishery sciences

Chromatographic techniques have been extensively utilized in fishery sciences for the separation and quantification of histamine and other compounds in food products. Methods such as gas chromatography (GC) (Staruszkiewicz 1981; Du et al. 2001), thin-layer chromatography (Ali et al. 2015), liquid chromatography (Fernandez and Mackie 1987, Veciana et al. 1995), and high-performance liquid chromatography (HPLC) (Hui and Taylor 1983, Vazquez et al. 1995, Hwang et al. 2003) have all been applied effectively to this end. A significant advancement in the application of GC was demonstrated in a collaborative study involving 21 laboratories, where a capillary column-based GC method was adopted as an official method by AOAC International and later designated as an AOCS-AOAC method by the American Oil Chemists' Society (Joseph and Ackman 1992). This method facilitated the analysis of encapsulated fish oils and ethyl esters, highlighting the utility of GC in lipid analysis. Further demonstrating the versatility of GC–MS, Katou et al. (1979) identified over fifty volatile compounds in fish samples using this technique. The study introduced an innovative sample preparation method involving the freezing of samples with liquid oxygen or nitrogen followed by fine pulverization, optimizing the analysis of volatile chemicals in fish tissues (Katou 1979). Another gas–liquid chromatographic method effectively detected methanesulfonate of *m*-aminobenzoic acid ethyl ester in fish muscle, underscoring GC's capacity for detecting specific chemical residues in biological matrices (Sills and Luhning 1977).

In terms of efficiency, a GC method described by Hwang et al. 2003 reduced histamine determination time in fish to under 20 min, presenting a rapid and effective alternative for the seafood industry. Similarly, Kim et al. (2020) developed a GC–MS method for the quantitative determination of phenol in fish tissues, which involves solvent extraction, derivatization, and subsequent analysis. This technique has potential for broader application to other biological matrices and phenol analogs, such as alkylphenols and chlorinated phenols (Kim et al. 2020). Additionally, Munir et al. 2021 utilized GC with flame ionization and mass spectrometry detectors to analyze biogenic amines in fish, while Qayoom et al. 2018 assessed organophosphate pesticide residues (Dimethoate) in Dal Lake, Jammu and Kashmir, using GC after liquid–liquid extraction (Qayoom et al. 2024a, b). These studies emphasize the broad applicability of GC in monitoring contaminants in aquatic environments.

Innovations in sample preparation and detection were further exemplified by (Chopra et al. 2019), who employed headspace solid-phase microextraction (HS-SPME) combined with GC–MS to detect polycyclic aromatic hydrocarbons (PAHs) in fish oil at part per billion concentrations. The method was developed using fish oil from capsules spiked with a standard PAH mixture, demonstrating its

Table 2 Comprehensive table: principles, components, detectors, applications, and findings of GC, GC–MS, and HPLC in fisheries research (Revised & merged Table 1 & 3)

Technique	Principle	Primary components	Detectors	Sample type	Key application	Findings	References
GC	Separates volatile compounds based on boiling point and interaction with the stationary phase	Injector, Column, Carrier Gas, Detector	FID: Hydrocarbons, fatty acids TCD: Dissolved gases, VOCs ECD: Halogenated organics, nitriles NPD: Organophosphates, nitrogen-based compounds SCD/NCD: Sulfur/nitrogen-containing analytes	Volatile compounds	Detection of hydrocarbons, pesticides, VOCs; assessment of fish freshness	Reduced histamine determination time in fish products (<20 min)	Hwang et al. (2003), Karim et al. (2012)
GC–MS	Combines GC separation with MS for compound identification using mass-to-charge ratio (m/z)	GC system coupled with Mass Spectrometer	MS: Trace organic pollutants, VOCs	Organic compounds, pollutants	Identification of trace pollutants, steroid hormones, VOCs related to fish spoilage	Efficient method for analyzing food flavor compounds and detecting pesticide residues in fish	Kim et al. (2020), Munir et al. (2021)
GC–MS/MS	Uses tandem mass spectrometry for enhanced selectivity and sensitivity in trace-level analysis	Triple Quadrupole Mass Spectrometer	MS/MS: Pesticides, environmental contaminants	Pesticides, contaminants	High-sensitivity detection of pesticide residues and environmental pollutants	Detection of ultra-trace levels of pesticides with minimized background ion interference	Krone et al. (2010)
HPLC	Separates non-volatile compounds using liquid solvent under high pressure	Pump, Injector, Column, Detector, Solvent	UV, Fluorescence, PDA: Antibiotics, lipids, vitamins	Non-volatile, polar compounds	Quantification of antibiotics, proteins, hormones, vitamins; analysis of carbohydrates and lipids	Detection of histamines, paralytic shellfish toxins, and microcystins in fish and fish products	Cicero et al. (2020), Qayoom et al. (2024a, b)
Reversed Phase HPLC	Separates based on hydrophobicity differences between analytes and the stationary phase	Reversed Phase Column, Solvent Reservoir	UV, Fluorescence: Vitamins, antioxidants	Antibiotics, lipids, vitamins	Analysis of purines, antioxidants, and pesticide residues in aquatic products	Improved detection of omega-3 fatty acids and mycotoxins in fish tissues	Roy et al. (2013), Bi et al. (2022)
Normal Phase HPLC	Separation based on polarity differences between the stationary and mobile phases	Normal Phase Column, Organic Solvent	UV: Polar compounds	Polar compounds	Analysis of fatty acids, vitamins, and antioxidants	Effective separation and quantification of bromophenols in seafood	Chung et al. (2003)
Size Exclusion HPLC	Separates molecules based on size using a porous stationary phase	Column with Porous Gel	UV: Macromolecules	Macromolecules	Determination of molecular weight distribution; identification of protein profiles in fish species	Adequate separation of water-soluble proteins for species identification	Armstrong et al. (1992)

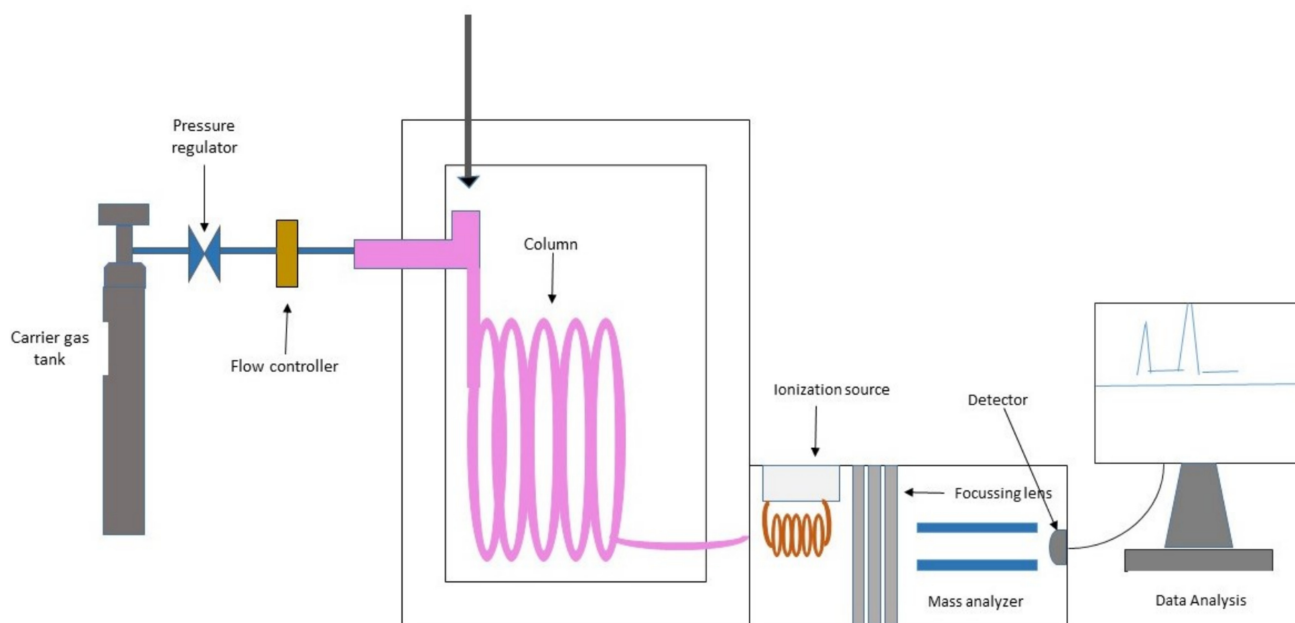


Fig. 2 Schematic of GC–MS workflow (Original design)

sensitivity and applicability to low-concentration analyses. Moreover, Wickrama et al. (2020) utilized high-resolution gas chromatography Orbitrap mass spectrometry to quantify halogenated polycyclic aromatic hydrocarbons (HPAHs) in the tissues of three tuna species. This study marked the first detection of substantial HPAH levels in biological samples, raising concerns about the potential health risks for consumers of these fish species.

These examples collectively underscore the critical role of GC and its variants in fishery sciences, particularly in the rapid and accurate detection of various compounds in food products and environmental samples. The ongoing refinement of chromatographic techniques continues to enhance their applicability and efficiency in the analysis of complex biological and environmental matrices.

In the evolving landscape of fisheries science, the future of gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS) appears promising with advancements poised to enhance sensitivity, specificity, and efficiency. Future developments will likely focus on improving detection limits and expanding capabilities for multi-residue analysis, enabling comprehensive screening of contaminants in fish tissues. Integrating metabolomics and lipidomics with GC–MS will deepen our understanding of fish metabolism and health, while automation and miniaturization trends will make these techniques more accessible for environmental monitoring and field applications. Collaborations across disciplines will drive innovation, supporting sustainable fisheries management, seafood safety assurance, and environmental stewardship in the face of global challenges.

Liquid chromatography

Liquid chromatography (LC) separates components of a mixture based on their affinity for a liquid mobile phase. Molecules with higher affinity elute faster, while those with lower affinity move more slowly through the stationary phase, resulting in delayed elution (Donato et al. 2012). The process as shown in Flowchart 2 involves preparing the stationary phase, injecting the sample into the mobile phase, and allowing the components to separate as the mobile phase moves through the stationary phase. An elution solution is used to complete the separation (Covey et al. 1986; Snyder et al. 2011; Fanali et al. 2017). Table 4 describes the separation techniques used in LC along with the characteristics.

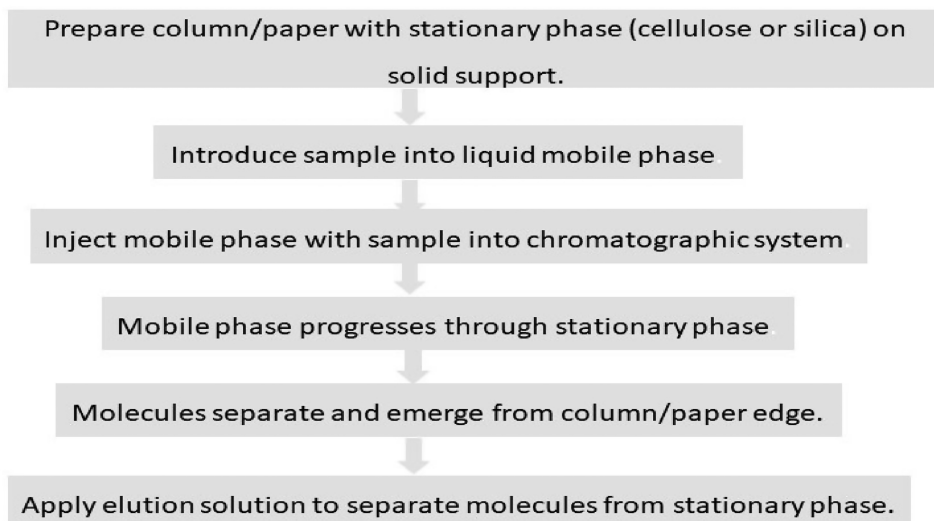
The Table 3 below provides an overview of different liquid chromatography methods, including their characteristics, typical applications, and mode of separation (Schematic view) (Figs. 3 and 4).

HPLC: high performance liquid chromatography

Principle/mechanism (Corradini et al. 1998; Hammood et al. 2023)

A sample compound is introduced into a column containing a porous stationary phase, while a liquid mobile phase is forced through the column under high pressure. A detector measures the retention time, which varies based on

Flowchart 2 Liquid Chromatography procedure



interactions between the stationary phase, analyte, and solvents. The movement of analyte is slowed by these interactions, with the extent of slowing determined by the properties of the analyte and the composition of the stationary and mobile phases (Broeckhoven et al. 2019) (Table 4).

Application of HPLC in fisheries

Fisheries science is essential for understanding and managing fish populations while ensuring the conservation of aquatic ecosystems. Within this field, fish toxicology is particularly significant, focusing on the effects of toxins and contaminants on fish health and the broader aquatic environment. Aquatic ecosystems are increasingly contaminated with a wide array of chemicals, including pesticides, heavy metals, industrial pollutants, and emerging toxins, which pose substantial risks to both fish and human health. Therefore, accurate detection and quantification of these toxins are crucial for evaluating their ecological impacts and potential health hazards (Rasheed et al. 2019). For instance, the residues of organophosphate insecticides such as Dimethoate and Chlorpyrifos in common carp muscle tissues have been assessed using High-Performance Liquid Chromatography (HPLC) coupled with a photodiode array detector (PDA) (Qayoom et al. 2024a, b).

Fisheries science covers various areas, including the assessment of fish quality and safety, identification of contaminants and toxins, evaluation of nutritional profiles, and monitoring of environmental pollutants. Among these, HPLC techniques have been extensively applied, leading to the development of efficient and reliable analytical methods. HPLC has emerged as a powerful analytical tool in fisheries science and fish toxicology due to its versatility, selectivity, and sensitivity (Justino et al. 2016). It effectively isolates, identifies, and quantifies a wide range of chemicals in fish

samples, from minute organic molecules to significant toxins and biomarkers (Fig. 5).

The application of HPLC in analyzing contaminants, toxins, and biomarkers has considerably enhanced our understanding of the impacts of environmental pollutants on fish and aquatic environments. When combined with other detection methods such as mass spectrometry (MS), HPLC provides a robust framework for identifying and quantifying toxins, including marine biotoxins, pesticides, herbicides, and pharmaceutical residues (Nimbkar et al. 2023). This capability is crucial for assessing toxicological risks and formulating appropriate mitigation strategies.

For example, the detection of paralytic shellfish poisoning (PSP) toxins has benefited from chromatographic techniques such as fluorescence detection (FLD) and mass spectrometric (MS) detection, which offer high sensitivity (Das et al. 2025). High-Performance Liquid Chromatography (HPLC) coupled with electrospray ionization tandem spectrometry (MS/MS) has shown promise as the most potent tool for PSP toxin identification, particularly for confirmatory analysis (Dell'Aversano et al. 2004). Furthermore, Lawrence 1987 suggested HPLC with fluorescent light detection (FLD) as an alternative method due to its sensitivity and full automation capabilities.

The primary chemical method for analyzing PSP toxins involves liquid chromatography (LC) combined with post-column oxidation and fluorescence detection, utilizing both isocratic and gradient elution modes. This method is essential for determining the limit of detection and quantification of PSP toxins in various shellfish samples across different matrices (Quilliam and Dell'Aversano 2001). The post-column method in HPLC, is highly regarded for its ability to allow simultaneous chromatographic separation of multiple samples (Abdul keyon 2014). This approach is invaluable in establishing toxin profiles based on factors like geographical



Table 3 Modes of separation and detectors in GC and LC

Technique	Separation mode	Explanation	Detectors	Applications in fisheries	References
Gas Chromatography (GC)	Partition-based separation using gas as a mobile phase and a stationary phase coated inside the column	GC separates compounds based on their boiling points and affinity for the stationary phase. Volatile compounds with low boiling points elute faster than others	FID: Organic compounds ECD: Halogenated organics TCD: Gases, VOCs MS/MS: Organic pollutants, VOCs	Monitoring of environmental pollutants, detection of pesticides, and analysis of lipid oxidation products in fish tissues	Kaur and Sharma, (2018), Rahman et al. (2021)
LC (HPLC)	Reversed Phase (RPC): Separation based on hydrophobicity between analytes and the stationary phase. (Fig a)	The stationary phase is nonpolar, and the mobile phase is polar, allowing hydrophobic compounds to elute later	UV, PDA: Proteins, antibiotics MS/MS: Lipophilic toxins, metabolites	Analysis of omega-3 fatty acids, lipophilic toxins, and pesticides	Gerssen et al. (2009), Qayoom et al. (2024a, b)
	Normal Phase (NPC): Separation based on polarity differences between analytes and phases	The stationary phase is polar, and the mobile phase is nonpolar, facilitating the elution of less polar analytes later	UV: Polar analytes	Detection of bromophenols, fatty acids, and vitamins in aquatic products	Chung et al. (2003)
	Size Exclusion (SEC): Separation based on molecular size using a porous stationary phase	Molecules are separated based on size; larger molecules elute faster as they are excluded from entering smaller pores in the stationary phase	UV: Macromolecules	Determination of molecular weight distribution and protein profiling for fish species	Armstrong et al. (1992)
	Ion Exchange (IEC): Separation based on ionic interactions	Charged analytes interact with oppositely charged stationary phases, and elution occurs with changes in ionic strength or pH of the mobile phase	Conductivity, UV: Ionic compounds	Analysis of amino acids and water-soluble ionic contaminants in fish tissues	Kataoka, (2023)
	Hydrophobic Interaction (HIC): Separation relying on hydrophobic interactions between analytes	Salt conditions influence analyte retention; compounds with higher hydrophobicity elute later	UV: Proteins	Analysis of hydrophobic proteins and contaminants in aquatic samples	Kataoka, (2023)
	Affinity Chromatography (AFC): Separation based on specific interactions between analyte and ligand	Analytes are retained based on biological specificity and eluted using changes in pH or ionic strength	UV, Fluorescence: Biologically active substances	Purification and concentration of bioactive compounds in fish tissues	Fanali et al. (2017)

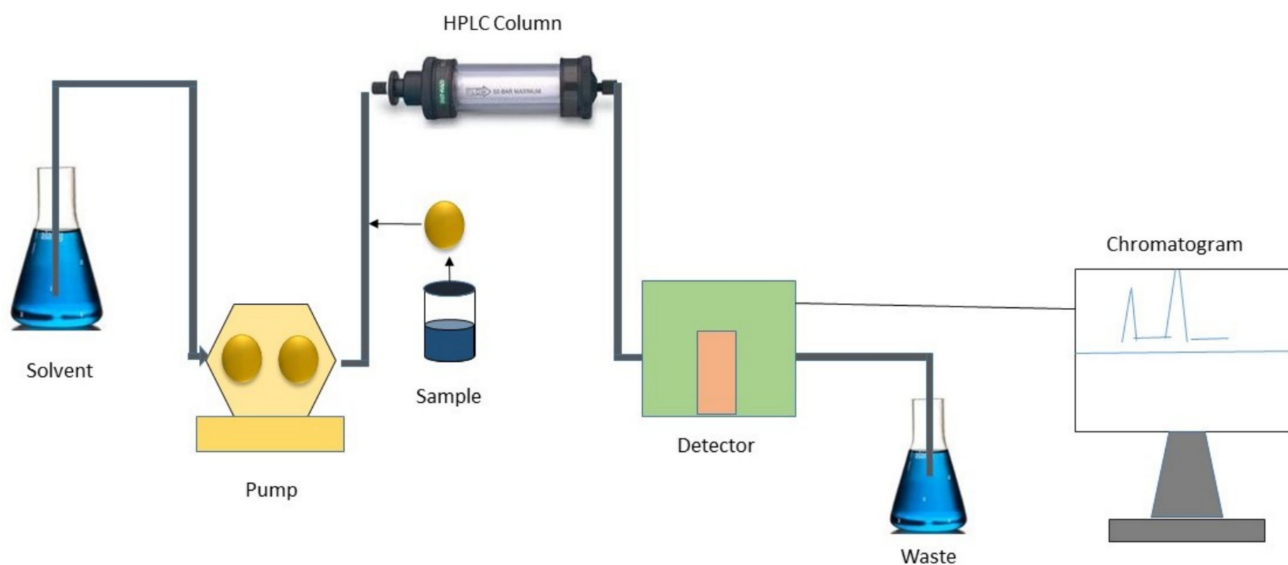
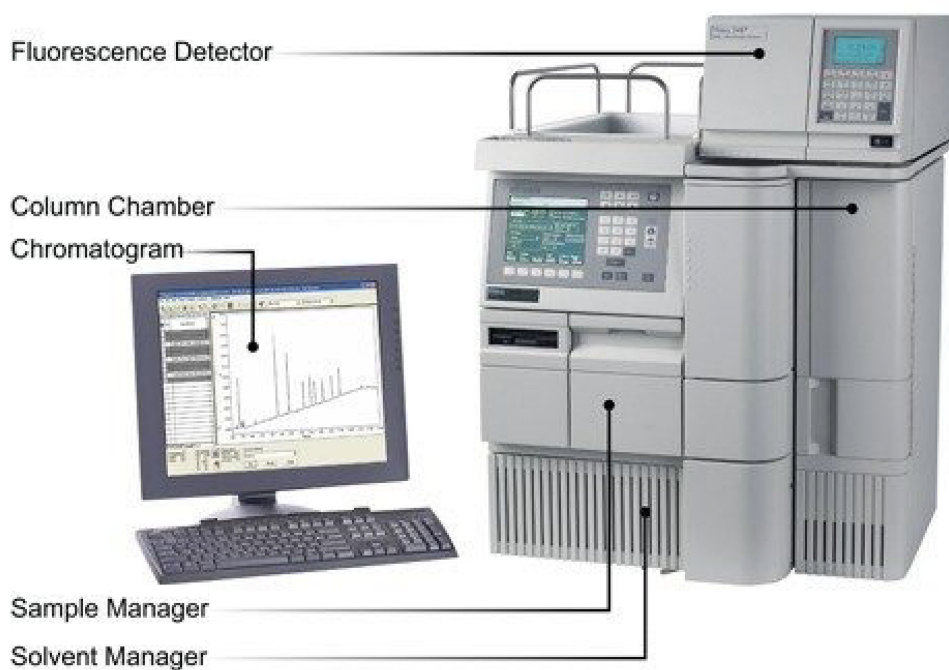


Fig. 3 Schematic Diagram of an HPLC system (original design)

Fig. 4 A typical HPLC system
(Source: www.waters.com)



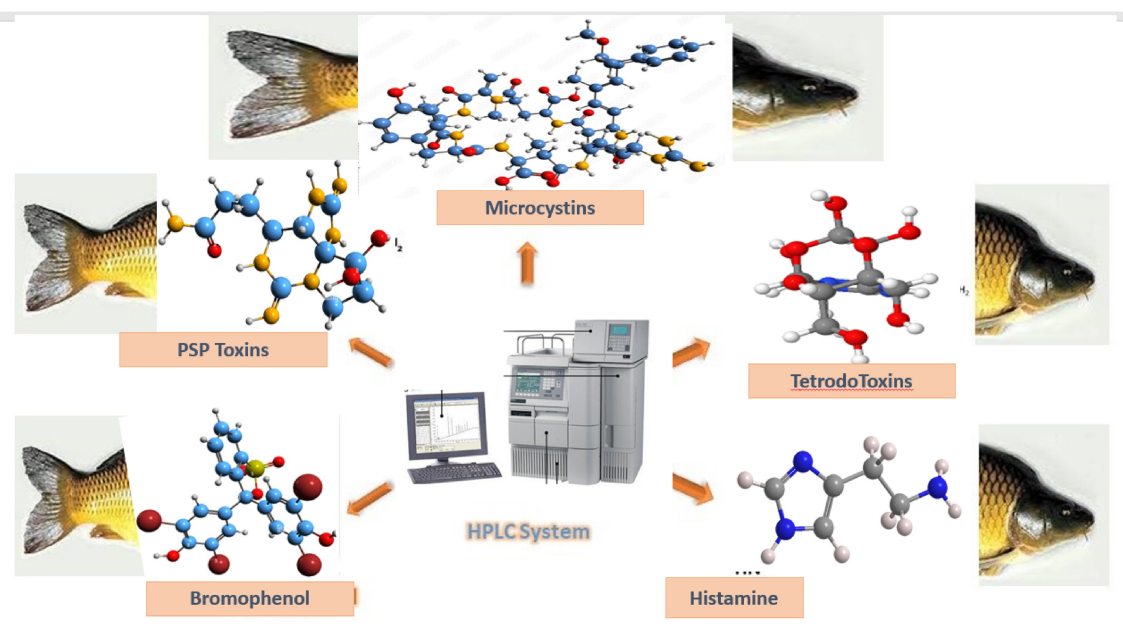
location, phytoplankton species, and shellfish species. Rey et al. further emphasized its utility in simultaneously separating and analyzing various PSP toxins in different shellfish varieties, including oysters, clams, mussels, and scallops, by adjusting the concentration of heptane sulfonate in the mobile phase. HPLC's ability to provide more reliable results compared to alternative methods like the mouse bioassay (MBA) underscores its importance in environmental monitoring and regulation (Yu et al. 2013).

In terms of biogenic amines (Zhang et al. 2021) a highly effective method has been developed for determining eight biogenic amines (BAs) in aquatic products and their derivatives using High-Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (HPLC–MS/MS) without the need for derivatization. The method involves sample extraction with a 5% perchloric acid solution followed by cleaning with n-hexane. Analyte separation is achieved using an ACQUITY UPLC HSS T3 column with



Table 4 Application of HPLC in detection of antibiotics in fisheries

Antibiotics		Characteristics	References
Sulphadimethoxine sodium Trimethoprim Florphenicol	Fish tissues	X-Terra™ C18 analytical column and the isocratic mobile-phase methanol: phosphate buffer (pH 3 ± 0.1) in ratio of (40: 60, v/v) UV detection	Riad et al. (2015)
Oxytetracycline	Salmon Fish muscle	HPLC mobile phase containing Mg(II) Fluorescence detection HPLC with UV detection	Coyne et al. (2004), Canada et al. (2009)
Sarafloxacin	Gilthead seabream	mobile phase consisted of a mixture of 30% acetonitrile – methanol (in a 3:2 ratio, v/v) and 70% trifluoroacetic acid 0.1% with pH adjusted to 2.15 Fluorescence detection with excitation at 278 nm and emission at 450 nm	Hasan et al. (2022)
Sulfonamides	Catfish Trout muscle	HPLC post-column derivatization fluorescence detection, HPLC MS/MS HPLC – DAD at 270 nm	Espinosa (2009), Dmitrienko et al. (2014)
Tetracyclines	Catfish	HPLC Fluorescence detection HPLC MS/MS	Pecorelli (2004), Dinh (2020)
Penicillins, Amphenicols	Gilthead seabream	The mobile phase was a mixture of aqueous solution of ammonium acetate 0.05 M and ACN, beginning initially isocratic and then ending gradient at 25 min	Agrahari et al. (2013)
Florfenicol	Channel catfish, Lake sturgeon, Rainbow trout	Reverse phase HPLC HPLC with UV detection	Vue et al. (2002)
Chloramphenicol	Yellow fish tuna	HPLC	Shen and Jiang (2005)
Furazolidone	Farm Fish	HPLC – UV	Hui and Taylor (1983)

**Fig. 5** Application of HPLC in fishery sciences

a gradient elution method, with the mobile phase composed of 0.5% formic acid and acetonitrile. The method demonstrates excellent linearity, with correlation coefficients (R^2) greater than 0.99 for all analytes. Sensitivity is high, with detection limits ranging from 0.1 mg/kg for tyramine, 2-phenylethylamine, and tryptamine to 1.0 mg/kg for spermidine, spermine, cadaverine, histamine, and putrescine. Recovery rates are robust, averaging between 70.9% and 113.1%, with relative standard deviations (RSDs) spanning from 0.33% to 10.81%, confirming the method's precision and accuracy for detecting biogenic amines in various aquatic products and derivatives (Tiris et al. 2023).

Histamine detection in fish and fishery products has also been extensively studied. For example, Jinadasa (Jinadasa et al. 2016) employed a method involving sample homogenization with trichloroacetic acid, followed by separation using Amberlite CG-50 resin and a C18-ODS Hypersil reversed-phase column. Detection was carried out using an excitation wavelength of 350 nm and an emission wavelength of 450 nm. A standardized HPLC–UV method, incorporated dansyl chloride as a pre-column derivatization reagent, followed by a liquid–liquid extraction purification procedure and separation using reverse-phase HPLC on a PerkinElmer Flexar HPLC system (Duflos et al. 2019). Additionally, Nadezhda introduced an analytical method for histamine detection using HPLC coupled with fluorescence detection, involving solid-phase extraction (SPE) and derivatization using o-phthalaldehyde (Nadezhda et al. 2012).

Recent advances include the development of a rapid and efficient Ultra-High-Performance Liquid Chromatography with Diode Array Detector (UHPLC–DAD) technique for histamine quantification in fish and fishery products (Cicero et al. 2020). This method achieves a runtime of just 6 min, with excellent validation parameters including a Limit of Quantification (LOQ) of 7.2 mg/kg, a Limit of Detection (LOD) of 2.2 mg/kg, and a recovery rate of around 100%. Similarly, Ayyub Kounoun presented an HPLC–FLD technique for quantifying histamine levels in various fish samples, eliminating the need for sample purification steps, and verified its accuracy and practical application (Lewis 1999; Kounoun et al. 2022).

Regarding tetrodotoxins, Chih-Yu Chen and Hong-Nong Chou employed HPLC to detect Tetrodotoxin (TTX) in Lined-Moon Shell and Puffer Fish (Chen and Chou., 1998). Their use of a fluorometric HPLC system proved highly effective for detecting TTX and related compounds, achieving an impressive 91% recovery rate (Li et al. 2023). Their research also demonstrated a strong correlation between results obtained from the mouse bioassay and HPLC analysis. Hasan et al. conducted the extraction and purification of tetrodotoxin from puffer fish liver using thin-layer chromatography and identified the toxin through various analytical

techniques, including IR, NMR, and mass spectrometry (Hasan et al. 2008).

Microcystins, potent toxins produced by cyanobacteria, also pose significant threats to aquatic ecosystems and human health. HPLC has emerged as a valuable method for separating and quantifying microcystins in fish samples. A tandem cartridge setup was used to extract and separate microcystins from fish tissue samples for analysis using HPLC with photodiode array detection (PDA) (Xie and Park 2007).

Furthermore, the detection of antibiotics like sarafloxacin using fluorescence detection and UV/Vis PDA, as well as the analysis of sulphonamides and tetracyclines in aquaculture (the chemical structure of which is given in Table 5, exemplifies the broad application of HPLC in fisheries science. Reversed-phase HPLC remains the predominant analytical approach for separating sulphonamides (SAs), with HPLC–MS being the primary tool due to its superior selectivity and sensitivity. Ultra-High-Performance Liquid Chromatography (UHPLC) has gained popularity for the rapid separation of SA compounds, with modern HPLC systems enhancing various aspects of chromatography, including speed, sensitivity, and resolution, while reducing matrix interference. This technology shows promise in rapidly screening multiple veterinary antibiotics, including SA compounds, in food and environmental samples (Dmitrienko et al. 2014). Recent research has also highlighted the detection of oxytetracyclines in various fish samples using UHPLC (Dinh et al. 2020). A study done by Singh and Joy 2008 examined immunocytochemical distribution and dynamics of vasotocin (VT) in the air-breathing catfish *Heteropneustes fossilis* concerning its reproductive cycle. Joy et al. 2014 conducted an in vitro study on the modulation of ovarian steroidogenic activity by catecholamines in the catfish *Heteropneustes fossilis* using HPLC. A high-performance liquid chromatography-electrochemical (HPLC–EC) detection method was employed to characterize estradiol-17 β (E2) and its metabolites (2-hydroxyE2, 4-hydroxyE2, and 2-methoxyE2) (Mishra and Joy 2006). The study also explored their seasonal variations and periovulatory changes in the ovary of the catfish *Heteropneustes fossilis*. One more study used HPLC and demonstrated the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis, in the ovary of the catfish to explore the potential physiological role of catecholamines in gonadal function (Chourasiya et al. 2010).

Overall, HPLC and its advanced variants continue to play a critical role in fisheries science, particularly in fish toxicology, by enabling the precise detection and quantification of contaminants, toxins, and biomarkers. This contributes significantly to our understanding of the impacts of environmental pollutants on fish and aquatic environments and



Table 5 Application of HPLC fishery sciences

S. No	Application	HPLC technique	References
1	<i>Quantification of toxins</i> <i>Histamines</i> <i>Paralytic shellfish poisoning toxin</i> <i>Microcystins</i>	Reverse phase HPLC UHPLC-DAD HPLC-FLD HPLC-PDA	Cicero et al. (2020), (2020), Jinadasa et al. (2016), Moreno (2005), Jinadasa (2016)
2	Quantification of antibiotics/pharmaceuticals	Reversed phase HPLC	Wong et al. (2021), Sigonya (2021)
3	Determination of tetrodotoxin	Reversed phase HPLC with Florescence detection	Turner (2014)
4	Analysis of purines in fish oils	Reversed phase HPLC with UV/Vis detection	Roy et al. (2013)
5	Determination of Antioxidants (Ethoxyquin)	Reversed phase HPLC with electrochemical detection	He and Ackman (2000), Kranawetyogl et al. (2019)
6	Analysis of lipophilic toxins (Okadaic acid)	Reversed phase HPLC with mass spectrometry	Gerssen et al. (2009)
7	Analysis of polyunsaturated fatty acids	Normal phase HPLC	Sajiki and Yonikubo (2002)
8	Determination of heavy metals	Chelation HPLC	Alpdogan et al. (2017)
9	Analysis of pesticide residues	Solid phase extraction with HPLC	Qayoom et al. (2024a, b)
10	Quantification of mycotoxins	Immunoaffinity column coupled with HPLC	Bi et al. (2022)
11	Detection of biogenic amines	HPLC–MS/MS without derivatization	Tiris et al. (2023)
12	Fish species identification using protein profiles	HPLC using reversed-phase and size exclusion columns	Armstrong et al. (1992), Seipke et al. (1986)
13	Analysis of flavor compounds in seafood (e.g., Bromophenols)	Reversed-phase HPLC with gradient elution and UV detection	Bordin et al. (2019)
14	Detection of microplastics and associated organic contaminants	Reversed-phase HPLC and HPLC–MS	Emerging research
15	Analysis of amino acids in fish muscle proteins	Ion-exchange HPLC	Ramachandran et al. (2019)
16	Study of environmental pollutants in aquatic habitats	Reversed-phase HPLC–MS	Yu et al. (2013)
17	Analysis of amino acids in fish muscle proteins	Ion-exchange HPLC	Ramachandran et al. (2019)
18	Determination of steroid hormones in fish plasma	HPLC with fluorescence or electrochemical detection	Martin et al. (2020)
19	Vitamin content analysis in fish tissues	Reverse phase HPLC with UV detection	Martin et al. (2020)

supports the development of effective strategies for environmental monitoring and regulation.

Detection of different antibiotics by HPLC

Bromophenols, recognized as essential flavor compounds in various seafood, including fish, shellfish, crustaceans, and algae, have garnered significant attention due to their role in the characteristic taste of these products (Chung et al. 2003). The RP-HPLC/UV technique, offers a promising approach for the effective separation and simultaneous quantification of specific bromophenols—namely, 2-bromophenol, 4-bromophenol, 2,4-dibromophenol, 2,6-dibromophenol, and 2,4,6-tribromophenol—within fish samples (Bordin et al. 2019).

This method's application of gradient elution and the use of varying wavelengths have been shown to significantly enhance analysis efficiency, reducing the overall time required for detection while maintaining a high level of sensitivity. Notably, the technique achieves remarkably low detection limits, which are crucial for accurately assessing bromophenol concentrations in complex seafood matrices. However, while the method demonstrates strong potential for routine analysis, its implementation may require further validation across diverse seafood species and varying environmental conditions to ensure robustness and reproducibility across different sample types. Moreover, the method's reliance on gradient elution might introduce variability in retention times and peak resolution, necessitating careful

optimization and standardization to maintain consistency in analytical outcomes.

Fish species identification using HPLC

A method for fish species identification using High-Performance Liquid Chromatography (HPLC) was introduced, leveraging a sophisticated setup with a Waters HPLC system. The system, comprising components such as dual model 501 pumps, a model U6K injector, and a model 484 tunable UV detector, was paired with a Hi-Pore RP-304 C-4 column from Bio-Rad Laboratories. The methodology involved a mobile phase mixture of 30% solvent A and 70% solvent B, both containing acetonitrile in 0.1% trifluoroacetic acid. The analytical process featured a gradient elution from 17 to 55% solvent B over 60 min, monitored at 280 nm (Armstrong 1992).

While this method demonstrates a comprehensive approach to fish species identification, certain aspects warrant critical consideration. The use of a 60-min gradient elution, although methodically sound, may not be the most efficient for high-throughput environments where rapid analysis is crucial. Additionally, the choice of a tunable UV detector set at 280 nm, though standard, may limit the detection of certain compounds that absorb more strongly at other wavelengths, potentially missing critical species-specific markers. Furthermore, the specificity and robustness of this method across a wider range of species and environmental conditions remain to be fully validated.

Similarly, the study offers an alternative HPLC approach, utilizing a Beckman System Gold HPLC system equipped with an online UV 167 detector. The methodology focused on analyzing water-soluble proteins using a linear gradient elution from 35% solvent A to 90% solvent B over 35 min. The use of a Biorad HiPore RP 304 C4 column ensured adequate separation, and the flow rate of 1 ml/min appears appropriate for maintaining resolution while minimizing analysis time (Seipke et al. 1986).

However, several critical factors need to be addressed. The UV detection at 230 nm may not be optimal for all protein types, particularly those with minimal absorbance at this wavelength, which could lead to incomplete protein profiling. The linear gradient elution, while effective in this context, may require further optimization to improve the separation of closely eluting species, particularly in complex protein mixtures. Additionally, the transformation of peak areas into percentages, excluding BSA area counts, might introduce bias in quantification, potentially skewing the comparative analysis of protein content across species.

While both (Seipke 1986, Armstrong 1992) present valuable methodologies for fish species identification using HPLC, there are areas where each method could benefit from

further refinement. Enhancements in detection sensitivity, elution efficiency, and broader validation across species would contribute to more robust and reliable analytical techniques in fisheries science. Table 5 represents the techniques used in HPLC for detection and quantification of various chemicals.

High-Performance Liquid Chromatography (HPLC) has indeed become a cornerstone in fisheries science, credited for its precision and versatility in analyzing a wide array of compounds, toxins, and contaminants. Its evolution from early chromatographic techniques to a highly sophisticated analytical method underscores its significant role in scientific research. However, while HPLC's contributions are well-documented, a critical examination reveals areas that require further scrutiny and potential improvement to maximize its application in fisheries science.

HPLC's historical development is rich, tracing back to the foundational work of Mikhail Tsvet and further refined by figures such as Archer Martin and Richard Synge. The technological advancements that followed, particularly in the 1970s with improvements in column packing materials, detectors, and automation, solidified HPLC as an essential tool in analytical chemistry. Despite this progress, it is important to recognize that HPLC, like any analytical method, is not without its limitations. These include challenges related to the high cost of equipment, the need for skilled operation, and the potential for variability in results due to differences in column performance or mobile phase composition.

In fisheries science, the role of HPLC in fish toxicology assessment, contaminant identification, and environmental monitoring is undeniable. Its sensitivity and selectivity make it invaluable for detecting toxins such as paralytic shellfish poisoning (PSP) toxins, histamines (Nadezhda et al. 2012), tetrodotoxin (TTX) (Turner 2014), and microcystins (Cicero 2020). For instance, HPLC's ability to quantify multiple toxins simultaneously, as noted in studies like those by (Cicero 2020 and Lewis 1999) significantly enhances the safety and quality of fishery products. However, the reliance on HPLC for these critical analyses also highlights potential issues, such as the need for extensive method validation across different matrices and environmental conditions to ensure accuracy and reliability. Moreover, the specificity of HPLC in detecting certain compounds could be influenced by the choice of detectors and mobile phases, which requires careful optimization for each application.

The application of HPLC in flavor analysis, specifically in separating and quantifying bromophenols in seafood, is another area where this technique excels. Sproll et al., demonstrated HPLC's effectiveness in this regard, contributing to the overall quality assessment of fishery products. Nevertheless, flavor analysis via HPLC can be complex, as the separation of closely related compounds often requires finely



tuned conditions, which may not be easily replicated across different laboratories or sample types (Louw 2021).

Additionally, HPLC's utility in generating protein profiles for fish species identification is crucial for fisheries management. The method developed by Armstrong et al. exemplifies HPLC's potential in this domain. Yet, the process of species identification through protein profiling is not without challenges, particularly in terms of the reproducibility of results and the potential interference from other proteins or sample impurities (Armstrong 1992). The necessity for highly purified samples and the precision required in peak identification underscore the importance of rigorous methodological control in such applications.

While HPLC stands out as a versatile and indispensable tool in fisheries science, its application is not without challenges. The high sensitivity and specificity that make HPLC valuable also demand meticulous method development and validation. The costs associated with HPLC, both in terms of equipment and operational expertise, must be weighed against its benefits. As fisheries science continues to evolve, the ongoing refinement of HPLC methodologies and the integration of complementary techniques will be essential to fully harness the potential of this powerful analytical tool.

Comparative analysis of GC, GC–MS, and HPLC in fisheries research

Sensitivity and specificity

When comparing the sensitivity and specificity of gas chromatography (GC), gas chromatography-mass spectrometry (GC–MS), and high-performance liquid chromatography (HPLC) in fisheries research, each technique offers unique advantages. GC, though effective for separating volatile compounds, has moderate sensitivity compared to GC–MS, which provides higher specificity and detection limits due to the addition of mass spectrometry. This makes GC–MS particularly valuable for detecting trace levels of organic pollutants, such as pesticides and hydrocarbons, in aquatic environments. HPLC, on the other hand, is highly sensitive for non-volatile and polar compounds, such as antibiotics, proteins, and hormones, offering exceptional accuracy in detecting contaminants that cannot be volatilized or degraded under high temperatures.

In fisheries applications, GC–MS stands out for its ability to accurately identify and quantify trace amounts of pollutants with superior sensitivity and specificity, whereas HPLC is preferred for its high precision in the analysis of large biomolecules and polar compounds.

Cost and practicality

From a cost and practicality perspective, GC and HPLC are relatively more affordable than GC–MS, as the latter requires a mass spectrometer, increasing both initial equipment costs and long-term maintenance. GC and HPLC systems are widely used, cost-effective techniques, with relatively simple setups, making them more accessible for routine analysis in fisheries research. HPLC also offers the advantage of handling a wider range of sample types with minimal sample preparation.

However, GC–MS, while more expensive, offers unparalleled specificity and sensitivity in detecting trace pollutants, justifying its cost in studies requiring highly precise measurements. Maintenance for all three systems includes regular calibration, cleaning, and replacement of components, though GC–MS tends to have higher upkeep costs due to the complexity of the system.

Suitability for different compounds

GC and GC–MS are best suited for volatile and semi-volatile compounds, making them ideal for analyzing pollutants such as pesticides, hydrocarbons, and volatile organic compounds (VOCs) that are common in fish tissues and aquatic environments. These techniques excel in the separation of compounds based on their boiling points and interaction with the stationary phase.

HPLC, however, is better suited for non-volatile and polar compounds that cannot be easily volatilized, including antibiotics, proteins, and hormones. In fisheries research, HPLC plays a crucial role in assessing the presence of such contaminants, particularly in studies focused on the physiological and biochemical impacts on aquatic species.

Each technique's suitability is determined by the nature of the analytes in question, with GC and GC–MS excelling in the analysis of volatile pollutants, and HPLC providing superior performance in the detection of non-volatile, polar compounds.

Future prospects of GC, GC–MS, and HPLC in fisheries research

Technological advancements

Emerging technologies such as automation and AI-driven data analysis are set to significantly enhance the performance and efficiency of GC, GC–MS, and HPLC in fisheries research. Automation is expected to streamline sample preparation, injection, and data collection, reducing manual errors and increasing throughput. In GC and

HPLC, automated systems can improve reproducibility, minimize the time required for analysis, and allow for real-time monitoring of environmental samples.

AI and machine learning algorithms are poised to revolutionize data interpretation. These tools can process large datasets generated by mass spectrometry, improving pattern recognition and enabling the identification of complex and previously undetected compounds. AI-driven analysis can also optimize method development, enhancing detection limits and accuracy for both GC–MS and HPLC, particularly in the context of trace contaminant analysis.

Potential research areas

Several unexplored applications of GC, GC–MS, and HPLC in fisheries remain open for future investigation. One promising area involves the comprehensive study of microplastic pollution in aquatic systems and its bioaccumulation in fish tissues. Currently, there is limited research applying these techniques to quantify microplastic-associated organic contaminants in fishery products, which could have significant implications for food safety and environmental health.

Another area ripe for exploration is the metabolic profiling of fish species under various environmental stressors, such as climate change, pollution, and habitat degradation. GC–MS and HPLC could be instrumental in metabolomics studies to assess biochemical changes in fish species, providing insights into their adaptive mechanisms and overall ecosystem health.

Interdisciplinary approaches

The future of fisheries research will increasingly rely on interdisciplinary approaches, integrating traditional analytical techniques with advanced methods such as bioinformatics, molecular biology, and genomics. Combining GC, GC–MS, and HPLC with bioinformatics can enable the comprehensive analysis of metabolic pathways, contaminant exposure, and their physiological impacts on fish species.

For example, coupling chromatographic techniques with genomic and transcriptomic data could offer deeper insights into how pollutants affect gene expression and metabolic function in aquatic organisms. This integration would allow for a more holistic understanding of the interactions between contaminants and biological systems, leading to better environmental monitoring and more effective conservation strategies.

In summary, as technological advancements continue to reshape the analytical landscape, GC, GC–MS, and HPLC are expected to evolve in their application and relevance,

particularly in fisheries research, offering new opportunities for discovery and environmental management.

Summary of findings

In this review, the comparative analysis of gas chromatography (GC), gas chromatography-mass spectrometry (GC–MS), and high-performance liquid chromatography (HPLC) highlights the unique advantages and limitations of each technique in fisheries research. GC is highly effective for separating and analyzing volatile compounds but offers moderate sensitivity compared to GC–MS, which enhances detection limits and specificity through its mass spectrometry capabilities. GC–MS excels in the precise identification of trace pollutants, making it a powerful tool in environmental monitoring and metabolomics. On the other hand, HPLC provides superior accuracy for non-volatile, polar compounds, such as antibiotics and proteins, which are crucial for assessing the impact of contaminants on aquatic life. However, HPLC is limited in its application to volatile compounds. While GC and HPLC offer cost-effective options, GC–MS demands higher initial investment and maintenance costs but justifies them with unmatched sensitivity and specificity.

Recommendations

For researchers selecting an appropriate method, the choice should depend on the nature of the target analytes and the specific research objectives. GC and GC–MS are recommended for studies focused on volatile and semi-volatile compounds, such as pesticides and hydrocarbons, especially where high sensitivity and specificity are required. HPLC is better suited for analyzing non-volatile, polar compounds like proteins, antibiotics, and hormones. For projects with budget constraints or routine analyses, GC or HPLC may suffice, while studies requiring trace-level pollutant detection or complex compound identification should prioritize GC–MS despite its higher cost. Researchers are encouraged to explore hybrid approaches and integrate emerging technologies, such as AI-driven analysis, to optimize results and enhance the accuracy of their findings in fisheries research.

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Declarations

Conflict of interest Authors declare that no conflict of interest exists.

Ethical approval Not applicable.

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