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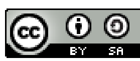
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Comparative Study of HPLC Detectors in PT. FIP: A Review

Annisa Luthfiyah Handini[1*], Patihul Husni[2], Indriana Agustin Claradila Frinda[3]
[1]Apothecary Professional Study Program, Padjadjaran University, Indonesia
[2]Department of Pharmaceutics and Pharmaceutical Technology, Padjadjaran University, Indonesia
[3]Department of Quality Control, PT. FIP, Indonesia

Address: Jl. Raya Bandung – Sumedang Km 21, Jatinangor, Indonesia
**Author correspondence:* annisa20022@mail.unpad.ac.id

Abstract. *High Performance Liquid Chromatography (HPLC) is a vital analytical technique used for separation, identification, and quantification of compounds in complex mixtures. This review explores the comparative performance of four detectors employed in the Chemical Laboratory of PT FIP: UV-Visible, Refractive Index (RI), Conductivity, and Evaporative Light Scattering Detector (ELSD). Each detector operates on distinct principles and is selected based on analyte characteristics such as the presence of chromophores, ionic nature, or volatility. The UV-Vis detector remains the most widely used due to its precision and compatibility with chromophoric compounds. However RI, Conductivity, and ELSD detectors serve as essential alternatives for analytes that lack UV absorption, non-ionizing, or exhibit low volatility. This review summarizes the key principles, advantages, limitations, and applications of each detector. The comparative evaluation highlights the strengths and constraints of each system, facilitating method optimization in pharmaceutical analysis.*

Keywords: *Conductivity, ELSD, HPLC Detectors, Refractive Index, UV-Vis.*

Abstrak. Kromatografi Cair Kinerja Tinggi (HPLC) merupakan teknik analisis penting yang digunakan untuk pemisahan, identifikasi, dan kuantifikasi senyawa dalam campuran kompleks. Tinjauan ini membahas komparasi kinerja dari empat detektor yang digunakan di Laboratorium kimia PT. FIP: Detektor UV-Visible, Indeks Bias (RI), Konduktivitas, dan Evaporasi Penghamburan Cahaya (ELSD). Setiap detektor bekerja berdasarkan prinsip yang berbeda dan dipilih berdasarkan karakteristik analit seperti adanya gugus kromofor, sifat ionik, atau volatilitas. Detektor UV-Vis tetap menjadi yang paling banyak digunakan karena presisi dan kompatibilitasnya dengan senyawa kromofor. Namun, detektor RI, Konduktivitas, dan ELSD berfungsi sebagai alternatif penting untuk analit yang tidak memiliki penyerapan UV, tidak mengionisasi, atau menunjukan volatilitas rendah. Tinjauan ini merangkum prinsip utama, keuntungan, keterbatasan, dan aplikasi setiap detektor. Evaluasi komparatif menyoroti keuntungan dan kendala setiap sistem, yang memfasilitasi pengoptimalan metode dalam analisis farmasi.

Kata kunci: Detektor HPLC, ELSD, Indeks Bias, Konduktivitas, UV-Vis.

1. INTRODUCTION

High Performance Liquid Chromatography or mostly called "HPLC" is one of the popular analytical methods. HPLC commonly used for chemical analysis includes identification, determination, or separation of mixtures samples based on affinity principle. The affinity implies the terms *"like dissolves like"* which means that the compound will dissolve in components with the same polarity (i.e polar in polar or non-polar in non-polar) (Abdu Hussen, 2022). The components can be either a mobile phase or stationary phase. In HPLC, a sample solution is injected into a column filled with a porous material (stationary phase), while

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a liquid (mobile phase) is pumped through the column at high pressure. As the mixture travels through the stationary phase, it separates into its individual components (Sadaphal & Dhamak, 2022). Substances with a higher affinity for the stationary phase move more slowly, retained longer on the stationary phase, and leading to a higher retention time whereas those with lower affinity elute faster through the column and resulting a shorter retention time. Based on this principle, there are two primary types of HPLC columns: normal-phase chromatography and reversed-phase chromatography (Fiorelia et al., 2022; Sunil, 2018).

Normal-phase chromatography has a polar stationary phase, typically composed of materials like silica gel which have a high specific surface area thereof non-polar molecules in mixtures sample being eluted faster and sooner than polar molecule. In reverse-phase chromatography, the terms *"reverse-phase"* to its operating principle, which contrasts with that of normal-phase. Reverse-phase consists of non-polar stationary phase and polar mobile phase. This type of HPLC column is most widely used in chromatography analysis due to their performance, selectivity, and practically. The example of non polar stationary phase is like C4, C8 (octyl), C18 (octadecyl), and octadecylsilane (ODS) column (Bhati et al., 2022; Fiorelia et al., 2022; Hashim, 2018).

In general, HPLC instrumentation consists of at least HPLC column, injector, pump, and detector (Ahmed, 2024). There are various types of detectors that can be used for HPLC. It depends on the sample being analyzed including the chemical properties of the analyte, possible impurities, sample matrix, sensitivity, detector availability, and cost. Most commonly used detector is ultraviolet-visible detector (UV-Vis). Not limited to that, other detectors such as PhotoDiode Array (PDA), fluorescence, Evaporative Light Scattering Detector (ELSD), etc can also be used in testing with HPLC (Sobolewska & Biesaga, 2025).

One of the pharmaceutical industries hereinafter referred as PT. FIP has four detectors consists of one existing detector and three new detectors that can be connected to HPLC instruments. The existing detector is UV-Vis and the new detectors are Refractive Index (RI), Conductivity, and Evaporative Light Scattering Detector (ELSD). This article aims to compare the new detector towards the existing detector used with HPLC instruments in Chemical Laboratories of PT. FIP.

2. RESEARCH METHODOLOGY

This review was conducted by gathering published articles relevant to specific keywords. The literature search was performed online using scientific databases such as Google Scholar, ScienceDirect, and NCBI.

3. RESULT AND DISCUSSION

High Performance Liquid Chromatography is a widely utilized analytical technique for identification, quantification, and separation of compounds within a mixture. HPLC process involves introducing a sample mixture into a column packed with a solid adsorbent material. A liquid pump continuously supplies the mobile phase into the column at a constant flow rate. As the mixture moves through the column, its individual components interact with the stationary phase to varying degrees, depending on their chemical structure, molecular size, and hydrophobic characteristics. The components only move within the mobile phase, so those that are more soluble in the mobile phase migrate more quickly, while those that prefer the stationary phase move more slowly. These differences in interaction facilitate the separation of the compounds, with each being identified by its specific retention time or the duration it takes to pass through the column. A detector is placed at the column outlet to identify the compound as they elutes. As the separation process is monitored by a recorder, a chromatogram is generated. Retention time serves as an indicator for qualitative analysis, while the peak area or height is used for quantitative analysis (Bachhav et al., 2023; Vidushi & Meenakshi, 2017).

HPLC Components

HPLC includes many components. The instrumentation is shown in Figure 1.

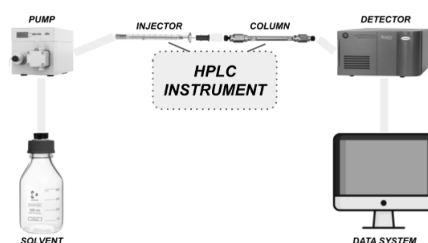

Figure 1. HPLC Instrumentation

In general, there are 4 main components namely (Abdu Hussen, 2022; Sah et al., 2021; Shukla et al., 2023):

1) **Injector**

The injector in an HPLC system is important to insert the sample to the system. The injector can be performed manually or automatically and designed to deliver liquid samples in volumes ranging from 0.1 to 100 µL with high precision and consistency. For high-throughput or repetitive analyses, an autosampler is often employed, offering automated injection and improved efficiency. Regardless of the method, the injector

must ensure consistent sample volumes and maintain chemical inertness to preserve analytical accuracy and reproducibility.

During the loading phase, the sample is injected into the loop injector using a syringe. In the injection phase, the sample is transferred from the loop to the pump for delivery into the column. As the injection process begins, an air rotates the valve to direct the solvent flow straight to the column, while connecting the injector needle to the syringe. The air pressure lifts the needle and position the vial directly beneath it. Finally, sample is intaken.

2) Column

Column is the most important part of chromatography that plays a role in the separation of analytes. Chromatography columns or also called as stationary phase can be polar or non-polar, depending on the type of column (normal-phase or reversed-phase chromatography). HPLC columns are typically made of polished stainless steel, ranging in length approximately 50 to 300 mm, with internal diameters between 2 and 5 mm. These columns are commonly packed with a stationary phase composed of particles measuring between 3 and 10 µm in size. For some samples, column heater may be needed. Column heater can keep the column at a certain temperature to ensure consistent results, even when analysis is conducted at room temperature.

3) Pump

In an HPLC system, the pump draws the mobile phase from the solvent reservoir and forces it through the column and detector at a consistent flow rate and pressure. The pump's primary function is to ensure a stable flow, typically between 1 to 2 mL/min, to achieve reproducible results across analyses. Operating pressure can range from 2000 to 5000 psi in standard operations, but depending on factors like column size, stationary phase particle size, flow rate, and mobile phase composition, pressures can reach up to 42.000 kPa (around 6000 psi).

Pump must be robust enough to maintain constant flow and pressure, as fluctuations in these parameters can lead to inaccurate results. Additionally, pumps can either maintain a constant mobile phase composition (isocratic) or gradually change (gradient), which impacts the elution time of sample components. The pressure at the injector can increase up to 20.000 kPa, influenced by mobile phase viscosity and other factors.

4) Detector

The HPLC detector located at the column's outlet, detects analytes as they are eluted from the chromatographic column. The role of the detector is to identify the individual molecules that exit the column, and then measure their quantity, enabling the chemist to perform a quantitative analysis of the sample components. The data from the detector is transferred to a recorder or computer, which generates a liquid chromatogram that represents the director's response. Each detector type provides a specific response, for the components separated by the column, ensuring the required sensitivity, and stability of fluctuations in the mobile phase.

The ideal characteristics of detector includes sensitive to specific compounds; robust stability and high reproducibility; quick response; less sensitive to variations in solvent, flow rate, and temperature; and non-destructive to the sample being analyzed (Abdu Hussen, 2022; Sah et al., 2021; Shukla et al., 2023).

These are four detectors used in Chemical Laboratory in the pharmaceutical industry of PT. FIP namely UV-Vis Detector, Refractive Index Detector, Conductivity Detector, and Evaporative Light Scattering Detector. The summary of differences between the four detectors is summarized in Table 1.

UV-Vis Detector

Ultraviolet – Visible detector is a detector for HPLC which uses a UV wavelength range of 200-400 nm and visible wavelength range of 400-800 nm. These detectors are used for chromophoric compound sample and provide selective responses based on the compound class or specific molecules, depending on the functional groups present in the eluting substances (Becket & Stenlake, 2007; Sunil, 2018).

Chromophoric is a compound contain chromophore part that absorbs UV or visible light (for example compound with carbonyl, carboxyl, or phenol group in the compound) UV-Vis absorption spectrophotometry based on the measurement of electromagnetic radiation attenuation by an absorbing substance. This radiation typically falls within the spectral range, differing from other regions in terms of energy and the type of molecular excitation. The observed attenuation may result from reflection, scattering, absorption, or interference (Passos & Saraiva, 2019; Wysocki, 2019).

In general, there are two main types of UV-Vis detectors: the fixed-wavelength (operate at a certain wavelength) and variable-wavelength (operate across a broader range of

wavelength). The result of the detection by UV-Vis are two-dimensional output for effective analysis (Cardoso et al., 2015; Dhole et al., 2012).

The principle of UV-Vis detector is based on Beer's Law with an equation follows:

$$\mathbf{A} = \boldsymbol{\varepsilon} \times \mathbf{b} \times \mathbf{c}$$

A = Absorbance

ε = Molar absorption coefficient ($M^{-1}cm^{-1}$)

b = Path length (cm)

c = Concentration (M)

The relation of each variable can be affected by various factors, including the performance and specifications of the spectrophotometer, photodegradation of the analyte, the presence of scattering or absorbing interferences, interaction between the analyte and the solvent, and variations in pH (Passos & Saraiva, 2019).

The UV-Vis detector is widely favored in analytical applications due to its numerous advantages including reliability, easy to use, high precision, and excellent linearity, making it particularly suitable for quality control of chromophoric compounds in pharmaceutical products. Although certain limitations exist such as the need for an optically transparent mobile phase and varying detector responses depending on the analyte's molar absorptivity, these challenges are typically minor and can be addressed through careful selection of mobile phase (Wysocki, 2019).

Refractive Index Detector

Refractive Index (RI) detector is the detector chosen for HPLC if the sample can not be detected by UV-Vis or fluorescent detector. This detector could detect compound like lipids, carbohydrates, or protein which do not have absorption at UV/Vis wavelength or can not emit fluorescent light (Al-Sanea & Gamal, 2022). The principle of this detector is by comparing the refractive index of the column effluent (which contains the analyte) with that of the pure mobile phase. Any analyte that elutes from the column generates a signal in the detector. While RI is often described as a universal detector, it does have some significant limitations. The detection limit is typically due to low sensitivity of detection, not compatible with gradient elution, and is highly sensitive to temperature variations (Reedijk et al., 2013).

The light refraction which is produced by detector is comprehensively described by Snell's Law (also known as Descarte's Law). This principle defines the relationship between the angle of incidence and the angle of refraction that occurs when light travels between two media with differing optical densities (Arygunartha & Ni Luh Putri Setyaningsih, 2022).

PT. FIP used The Waters® 2414 Refractive Index (RI) Detector that provides the highest sensitivity, stability, and reproducibility for quantification of components with limited or no UV absorption such as alcohols, sugar, saccharides, fatty acids, and polymers. This detector measures by detecting the changes in refractive index of eluent from the column compared to the mobile phase. RI are used to detect compounds that have no absorption in UV/Vis region while UV-Vis detector measures the compound contains chromophoric groups at specific wavelengths of UV-Vis.

Conductivity Detector

Conductivity Detectors (CDs) is a detector for ion chromatography and operates by measuring the conductivity of eluent which changes due to the elution of charged analyte (ionized compound with no chromophore groups such as Na^+, K^+). When a charged analyte is eluted, it alters the conductivity and triggers a detector signal (Bhirde et al., 2021; Knol et al., 2021).

Conductivity detectors typically offer moderate sensitivity (in nanogram range) and a reliable linear range. However, since only ionized compound can be detected and a conductive eluent is required, their application is mostly limited to aqueous chromatography system. For non-ionic analytes, indirect detection methods using conductive solvents can be developed (Bhirde et al., 2021; Knol et al., 2021).

Electrical conductivity is present in electrolyte solutions and is represented by Ohm's Law with equation below:

$$\mathbf{V} = \mathbf{I} \times \mathbf{R}$$

Ohm's Law describes the relationship between voltage (V), current (I), and resistance (R) in an electrical circuit. In the context of conductivity detection in HPLC, Ohm's Law is used to measure the electrical conductance (G) which is the reciprocal of resistance (G = 1/R). Conductivity detectors apply a voltage across electrodes in contact with the mobile phase and measure the resulting current to determine conductance. This conductance is directly related to the concentration of ionic species in the eluent. The basic equation for conductivity (k) is (Haddad, 1990):

$$\mathbf{k} = \mathbf{G} \times \mathbf{K}$$

k = Conductivity (S/cm)

G = Measured conductance (S)

K = Constant (cm^{-1}) [*)]

[*)] the constants value depends on geometry of the detector cell

PT. FIP used The Waters® 2432 Conductivity Detector that provides a highly sensitive and stable performance when performing non-suppressed ion chromatography. This detector measures the electrical changes of mobile phase as it passes through the detector. Conductivity detector are suitable for ion chromatography due to the respond to ionic compounds.

Evaporative Light Scattering Detector

Evaporative Light Scattering Detector (ELSD) is one of various detector for HPLC. HPLC with ELSD is a versatile detector capable of identifying both semi-volatile and non-volatile analytes across a broad dynamic range. This detector has been extensively used for analysis of pharmaceutical products if the sample lack of chromophore groups (such as sugar and lipids) (Han et al., 2013; Piechocka et al., 2023). The stability and sensitivity of detector, and the absence of complex sample preparation serves ELSD as an alternative detector used for HPLC (Soyseven et al., 2022).

ELSD operates through a nebulization, evaporation, and light scattering steps. In the first step, nebulization, the mobile phase is converted into fine droplets and carried by an inert gas into the evaporation chamber. After that, the evaporation step occurs and the solvent must be fully removed from the droplet, leaving only the analyte particles for subsequent detection. The last step is detection through light scattering by the solid particles and measures the interaction of it between angles (relative to the incident beam) (Shock et al., 2011).

The ELSD are compatible with the gradient method and easier to use than others detector. ELSD also had greater robustness but less sensitive than other detectors with similar principles (Mousli et al., 2022). The corelation between the signal and the analyte mass in ELSD is non-linear. In these methods, the relationship between it can be represented by the equation follows (Vervoort et al., 2008):

$$\mathbf{A} = \mathbf{a} \times \mathbf{M}^{\mathbf{b}}$$

A = Area responses of the detector

M = Mass of the analyte

b = constants*)

*) the constants value depends on analyte and chromatographic condition.

PT. FIP used The Waters® 2424 Evaporative Light Scattering (ELS) Detector that specifically for analysis compounds with poor or no UV/Vis responses and analytes that do not ionize well in mass spectroscopy. This detector measures light scattered from particles in a vaporized sample. ELSD is less sensitive than UV-Vis detector but has a wider range because suitable for compounds that do not absorbs UV/Vis light or lack of chromophores.

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Table 1. Summary of Differences between The Existing and The New Detector in PT. FIP

Parameter	Existing Detector	New Detectors		
	UV-Vis	Refractive Index	Conductivity	Evaporative Light Scattering
Type		The Waters® 2414 Refractive Index (RI) Detector	The Waters® 2432 Conductivity Detector	The Waters® 2424 Evaporative Light Scattering (ELS) Detector
Purpose & Principles	Measures the absorbance of light at specific wavelength which has specific detection range (usually 200-800 nm).	Measures by comparing refractive index of eluent from the column (contains analyte) to the mobile phase.	Measures conductivity of eluent as it passes through the detector.	Measures light scattered from particles in vaporized sample.
Analyte	Chromophoric compound.	Compound with limited or no UV absorption (e.g lipid, carbohydrates).	Ionized compound with no chromophore group (e.g Na^+, K^+).	Compound do not absorbs UV-Vis light or lack of chromophores.
Advantages	1. Easy to use 2. High precisions 3. Excellent linearity	Universal detector	1. Can be used for ionic compound or charged solution 2. Sensitivity	1. Compatible with gradient method 2. Easy to use compare to detector with the same principle 3. Greater robustness
Limitation	1. Need optically transparent mobile phase 2. Detector responses depending on analyte's molar absorptivity	1. Low sensitivity of detection 2. Not compatible with gradient elution 3. Highly sensitive to temperature variations	Sensitive to changes of conductivity components.	Less sensitive than other detector with similar princple.

4. CONCLUSIONS

HPLC continues to be an indispensable technique in pharmaceutical quality control due to its high sensitivity, reproducibility, and analytical precision. At PT. FIP, four types of detectors – UV-Vis, RI, Conductivity, and ELSD – are employed to support diverse analytical needs. While UV-Vis detectors offer excellent performance for compounds with chromophores, RI detectors provide an alternative for non-UV absorbing substance such as sugar and lipids. Conductivity detectors are highly effective for ionic species, and ELSD serves as a robust option for compounds that neither absorb UV light nor ionize. Each detector type brings unique benefits and limitations depending on the physicochemical nature of the analytes. Therefore, understanding the principles and capabilities of each detector is crucial for selecting

the most appropriate system to ensure accurate, efficient, and comprehensive analysis. This comparative review supports method development and optimization efforts in pharmaceutical laboratories seeking to expand their analytical versatility.

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