HOW TO GUIDE

HPLC: Choosing the Right Detector

High performance liquid chromatography (HPLC) is a separation technique used in many industries, such as food safety, cannabis/hemp production, pharmaceutical development, manufacturing and quality analysis and control (QA/QC). The technique relies on a mobile phase and a stationary phase to separate components within a mixture. A high-pressure pump moves the mobile phase through the system. Molecules with a higher affinity for the mobile phase will migrate through the column more rapidly and interact less with the stationary phase. Once separated, a detector measures the concentration of the analytes and converts them into electrical signals; the concentration of each component is proportional to the amount that was eluted from the column. The time taken between injection and detection – known as the retention time – is specific for a given set of chromatographic conditions and may be compared with a standard for identification.[1,2] Various configurations exist for liquid chromatographs, however the highest efficiency separations are achieved using ultra-HPLC (UHPLC) as it offers greater chromatographic resolution and higher sensitivity, the ability to use <2 µm particle size columns, as well as requiring less time due to faster analysis.[3,4] The evolution to UHPLC, was ultimately driven by a demand for higher resolution separations of more complex and challenging samples.

With reference to applications using PerkinElmer LC 300™ HPLC/UHPLC System, this guide will highlight the different detectors available and provide tips to help you choose the most appropriate detector for your liquid chromatography application.

What to Consider When You Are Choosing HPLC Detectors

Detectors for HPLC/UHPLC determine the identity and concentration of eluting compounds in the mobile phase, and they can be categorized into two types: specific detectors and bulk property detectors. As the name suggests, specific detectors respond to specific compounds and their response is not dependent on the composition of the mobile phase. UV/Vis detectors are the most common examples of specific detectors, as they respond to compounds that absorb UV or visible light at particular wavelengths.5 Bulk property detectors, by contrast, are the most universal detectors for HPLC. They measure properties common to all analytes by measuring differences in the mobile phase with and without the sample. The universal nature of bulk property detectors places an increased emphasis on the selectivity of the chromatographic column, however they are limited in their sensitivity.[5,6] The refractive index detector (RI) is the most common example of a bulk property detector.

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Most of the detectors used in HPLC are non-destructive, making the method more attractive for purification or preparative work compared with detection such as LCMS or other chromatographic techniques such as gas chromatography. The non-destructive nature of detection also permits multiple forms of detection to be plumbed together in a serial fashion for greater insights. Each type of detector differs with regards to its sensitivity, specificity, selectivity and linear dynamic range, therefore the choice of detector is driven by the method goals for the application.[7] There are many characteristics to consider when choosing a detector and no one detector will have all of these properties, therefore several detectors have been developed over time to address particular challenges. It is important to consider the chemical nature of the analytes of interest and any potential interferences, when choosing a detector. Additionally, the combined use of complementary or orthogonal detectors is gaining popularity in drug discovery and other screening applications, where complementary information can be generated by separate detectors.[5]

UV/Vis Detectors

Ultraviolet visible (UV/Vis) detectors are the most popular, especially in quality control laboratories, due to their reliability, ease of use and universal response to chromorphoric compounds – which include most pharmaceuticals. While traditional applications of UV/Vis spectroscopy include materials characterization, optics, coatings, glass and color control, the technology has long been a standard for HPLC detection given its well-known performance attributes. There are essentially three categories of UV/Vis detectors: single or fixed wavelength detectors, variable or multi-wavelength detectors and diode array detectors (also referred to as photodiode array detectors). The sample concentration is determined by the amount of light absorbed by the sample. Single or fixed wavelength detectors rely on monitoring distinct wavelengths typically by employing a monochromator, whereas most variable or multi-wavelength and photodiode-array detectors rely on simultaneously gathering data at multiple wavelengths.[5]

When to Use UV/Vis Detectors

UV/Vis detectors are ideal for researchers working in regulated industries that process a large number of samples, or require a wide range of application flexibility, as they can quantify low-level impurities in the same run as the analyte of interest, delivering reliable results across a wide range of compounds with minimum operator training and low operating costs.[8] Choosing a UV/Vis detector will likely be based on factors such as wavelength range, sensitivity, sample type and sample size, current and future applications, required accessories amongst others.[9]

The Criteria to Consider When Choosing a Detector for HPLC/UHPLC:

- High sensitivity
- Reproducibility
- Predictable specificity
- Wide linear dynamic range
- Reliable and convenient
- Non-destructive
- Need for qualitative and/or quantitative information on detected compounds
- Fast response/fast data rates
- Availability and cost
- Compatibility with HPLC/UHPLC systems

Single or fixed wavelength UV/Vis detectors, considered the backbone of early HPLC systems, are cheap, simple and used for the majority of chromatographic applications that require UV detection.[5,10] An example of an application using a UHPLC with single UV/Vis detection is the analysis of patulin in apple juice. Patulin is a toxic substance produced by molds that are found on fruits, vegetables, cheese and grains. Despite its low potency, various studies demonstrating its genotoxicity have suggested that it may be carcinogenic. Conventional methods for patulin testing include multistep liquid-liquid extraction (LLE) or solid-phase extraction (SPE), yet these approaches require multiple steps for sample preparation and some use large quantities of solvents and chemicals. In an effort to develop a simple, robust and reliable method for patulin analysis in apple juice, one study used a PerkinElmer UHPLC system configured with a UV/Vis detector. The analyte was well separated from its other components in under five minutes, with results showing excellent retention time repeatability, as well as very good linearity (over the tested concentration range). This application can thus be considered an effective method for monitoring of Patulin in apple juice and covers the EU's maximum allowable limit of 10 µg/kg (10 ppb) for small children, sitting well below the overall 50 µg/kg (50 ppb) limit currently recognized by the FDA. An example of a schematic for a single or fixed wavelength detector is shown in Figure 1.[5]

Multi- (or variable) wavelength UV/Vis detectors (MWD) can be programmed to operate at the absorbance maximum of an analyte (if previously known) or to collect data at more than one wavelength to provide more insights and characterization. In a variable wavelength detector, light from a broad-spectrum lamp is directed through a slit to a diffraction grating that spreads the light out into its constituent wavelengths.

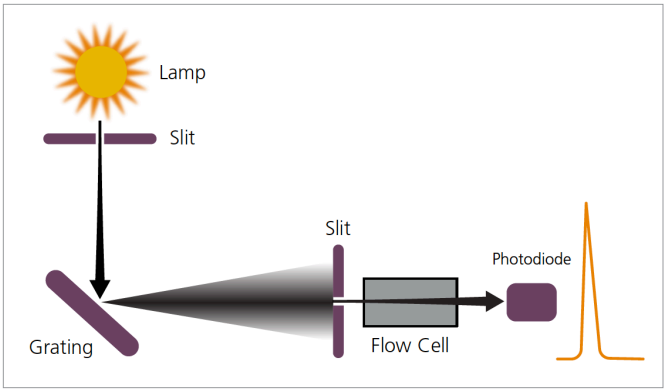

Figure 1. Typical schematic for a single or fixed wavelength UV detection. Adapted from Swartz, 2010.

MWD detection has been used in conjunction with HPLC to analyze common preservatives – known as parabens – that are used as antimicrobial preservatives in many everyday products, such as cosmetics, personal care products, food and pharmaceutical preparations. Parabens are absorbed by the skin without being broken down by esterase enzymes and studies have demonstrated that they can cause disturbances in the endocrine system and are associated with a range of effects, including an increased risk of breast cancer. It is therefore important that manufacturers producing paraben-containing products have robust analytical tools to consistently maintain regulatory and safety compliance. While the FDA does not require amounts to be listed on the labels of cosmetic products, they do require accurate labeling of ingredients. Therefore, in one study, eight personal care products were analyzed – two of which claimed to be paraben-free – to ensure that the analytes detected matched the list of ingredients on each product. Chromatographic separation was achieved using the PerkinElmer LC 300 HPLC System consisting of an LC 300 HPLC Pump, and an LC 300 HPLC Autosampler equipped with an integrated column oven – an LC 300 MWD Detector was used for detection. This method provided excellent chromatographic resolution between all closely eluting peaks, and allowed for monitoring of all preservatives at their optimum wavelength for optimum method sensitivity. The analytical results were consistent with the ingredients labels of each sample, with all preservatives found to be below the maximum concentration limit set by the EU Commission Regulation. This method provided excellent chromatographic resolution between all closely eluting peaks and the analytical results were consistent with the ingredients labels of each sample, with all preservatives found to be below the maximum concentration limit set by the EU Commission Regulation.[11]

PDA Detectors

The optical path of a photodioide array (PDA) detector is modified compared to a single or fixed wavelength detector. As shown in Figure 2, the light passes through a flow cell before it hits the grating and is spread across an array of photodiodes instead. PDAs extend the utility of UV/Vis detection by providing a spectrum for eluting peaks that can be used to aid in peak identification or to monitor for co-elution (which is beneficial during method development).[5] A spectrum can essentially provide a fingerprint of a compound that can then be compared to those in a library for positive identification. A spectrum can essentially provide a fingerprint of a compound that can then be compared to those in a library for positive identification. Spectral processing tools such to determine peak purity, wavelength max and absorbance ration are also available. Offering advanced optical detection with exceptional chromatographic and spectral sensitivity, they are perfect for the identification of unknown compounds or for ensuring complete component separation even under extreme conditions.[12]

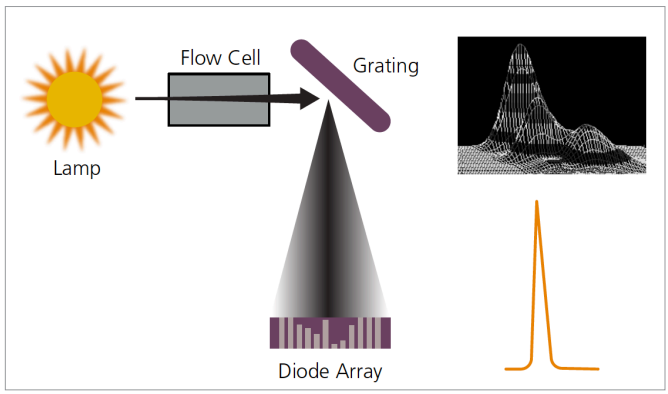

Figure 2. A schematic showing PDA detection. Adapted from Swartz, 2010.

When to Use PDA Detectors

One application of HPLC with PDA is cannabis research; as the use of cannabis products increases with state-level recreational legalization, cannabinoid compounds must be quantified using complete and robust methods. A failure to do so can result in negative health impacts, as well as a loss of consumer confidence. The analysis of the cannabinoid content in commercially available cannabis flower and fortified products – such as foods – depends on liquid chromatography to ensure label claims in product content descriptions are accurate.

Interestingly, studies of edible cannabis products purchased from licensed dispensaries in California revealed that only 17% of the 75 products purchased were labeled accurately, with the remaining either under-labeled (23%) or over-labeled (60%) with respect to THC content. Using the PerkinElmer LC 300 HPLC system (consisting of an LC 300 10k psi Pump and LC 300 Autosampler equipped with an integrated column oven) and an LC 300 PDA detector, one study analyzed 16 cannabinoids in under seven minutes. The results exhibited very good retention time repeatability, as well as excellent linearity over the tested concentration ranges, demonstrating that the PerkinElmer LC 300 HPLC with PDA detection is a fast and robust method for chromatographic separation and quantification.[13]

A second study used PDA detection to quantify isoflavones concentrations in soy nutraceutical supplements. Isoflavones are a class of naturally occurring water-soluble compounds found in several plants and food sources. They are structurally similar to estrogen and therefore actively bind to estrogen receptors in the body. While the estrogenic effects of phytoestrogens are typically weaker than natural estrogen, they can have a broad effect on human health; some studies have found that phytoestrogens exacerbate existing thyroid disorders, whilst others have found them to reduce the risk of certain cancers and prevent the onset of osteoporosis. Since various isoflavone supplements are available on the market (as nutraceuticals), verifying label-claim accuracy is crucial during the development and manufacturing of nutraceutical products to protect consumers. The results from this study, were consistent with the label-free claim for each sample, demonstrating that the PerkinElmer LC 300 18K UHPLC system with PDA detection is a robust and rapid method for the separation and quantification of soy isoflavones. This method poses a significant advantage over traditional analytical methods that are used in most highthroughput laboratories – and which consume a significant number of solvents.[14]

Fluorescence Detectors

Fluorescent detectors (FL) measure the optical emission of light by solute molecules after they have been excited at a high energy wavelength. Their specific excitation and emission wavelength profile aids in the characterization of individual components. FL detectors are incredibly specific, sensitive and selective detectors, offering greater sensitivity than UV/Vis detectors. In contrast to UV/Vis detectors which measures light that has been absorbed by the sample, FL detectors detect fluorescence emitted in an orthogonal direction to the exciting light. As illustrated in Figure 3, they are similar to single or fixed wavelength detectors, however two monochromators (or filters) are used (one to select excitation wavelength and the other to select emission wavelength).[5,15]

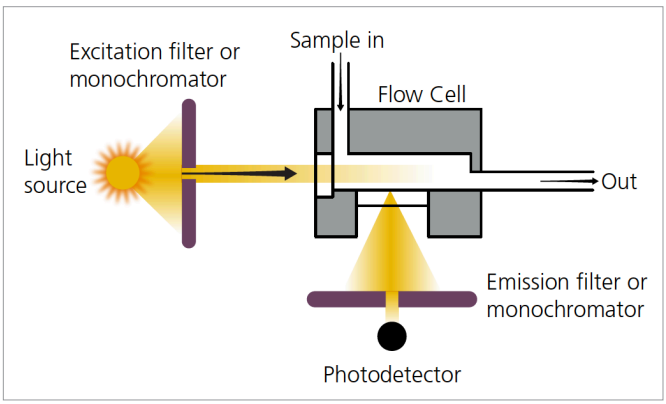

Figure 3. A schematic showing fluorescence detection. Adapted from: chromatographyonline.com.

When to Use FL Detectors

FL detection is ideal for a range of applications, including serum and plasma analysis for pharmaceutical research, environmental analysis of toxins and pollutants, vitamin analysis and the analysis of food and beverage degradation products.[16] With reference to environmental research, awareness of polycyclic aromatic hydrocarbons (PAHs) and their impact on public health has become increasingly prevalent. PAHs are generated by the combustion of fossil fuels and are found as a mixture of compounds that differ in behavior, environmental distribution and biological effects. Lower molecular weight PAHs, such as naphthalene, are water soluble and cause significant acute toxicity in invertebrate aquatic organisms. In contrast, high molecular weight PAHs bio-accumulate in aquatic organisms such as oysters, rainbow trout and zooplankton. Their low solubility in water means that they bind strongly to soils, sediments and particulate matter. An example of a high molecular weight PAH is benzo(a)pyrene, a by-product of incomplete combustion or the burning of organic material (cigarettes, gasoline and wood) – it is considered highly carcinogenic.

To protect the public from the carcinogenic effects of PAH exposure, the EPA has developed ambient water quality criteria. Researchers used the PerkinElmer UHPLC System with PDA and FL detectors to explore the levels at which 19 PAHs in surface water can be monitored. The results exhibited exceptional linearity for each PAH over the tested concentration ranges and – while none of the analyzed surface waters showed a detectable amount of PAHs – the naphthalene and benzo(a) pyrene spike recovery analysis demonstrated that UHPLC is a reliable and robust tool for the detection of PAHs at ppb levels.[17]

Refractive Index Detectors

The refractive index (RI) detector is the original and oldest detector used for LC and is revered due to its truly universal detection capabilities. RI detectors measure the difference in optical refractive index between the mobile phase and the sample. As can be see from Figure 4, there is a reference cell and sample cell. The reference cell is purged with mobile phase prior to analysis where a valve is used to then direct the column effluent through the sample cell. As light passes through the two detector cells it is refracted differently, measured by a pair of photodiodes that convert the signal to a measurable output voltage. While long ago they were considered a chromatographic mainstay, RI detectors are limited in comparison to other detection techniques due to a lack of sensitivity and gradient incompatibility issues.[5,18]

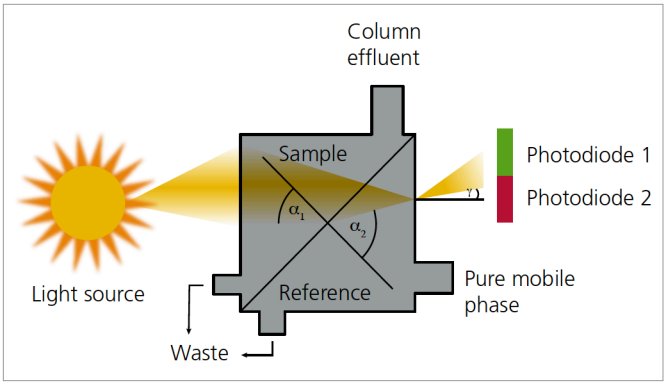

Figure 4. A schematic illustrating RI detection. Adapted from researchgate.

When to Use RI Detectors

RI detectors are best suited for the detection of samples with limited or no UV absorption. Some features to consider in a HPLC RI detector include temperature control to improve baseline stability and signal to noise performance. Since no chromophore on the analyte molecule is required, this detection method is used very successfully for the analysis of sugars, alcohols, fatty acids, carbohydrates and organic acids.[15, 18] Sucrose is the primary sugar found in maple syrup. The total sugar content of maple syrup is therefore typically based on the amount of sucrose and does not consider other sugar sources such as glucose, fructose and complex carbohydrates. HPLC is commonly used to analyze simple sugars, specifically hydrophilic interaction chromatography (HILIC) with refractive index (RI) is often used for sugars as they are polar compounds and lack a chromophore for UV detection.

In a study looking to develop a simple HILIC-RI method to analyze four common sugars found in maple syrup, scientists used the PerkinElmer LC 300 HPLC System (consisting of an LC 300 10K psi Pump, LC 300 Autosampler and integrated column oven) coupled with an LC 300 RI Detector. In addition to exhibiting good retention time repeatability and excellent linearity over the tested concentration range, this method effectively identified specific analytes contained in each of the syrup samples and compared their sugar profiles, both chromatographically and quantitatively.[20]

Conclusion

In summary, the decision on which detector to use for HPLC depends on a variety of factors. LC detectors based on the absorbance UV/Vis light are the most popular detectors due to their simplicity, reliability, sensitivity, and response to a wide range of sample compounds (if the analytes have sufficient UV absorbance for detection). FL detectors are more selective and can be more sensitive, however compounds must fluoresce to be detected. Based on UV/Vis principles, PDA detectors measure absorbance over a wide range of wavelengths and are therefore able to give UV spectra as well as chromatographic data to facilitate qualitative and quantitative analysis. For compounds that do not absorb UV light, RI detection is a simple and effective method.[18, 19, 21] The PerkinElmer LC 300 HPLC/UHPLC Systems[22] can be used in conjunction with a variety of different detectors, enabling a flexible and reliable workflow regardless of your application needs.

References

1. Ibrahim D, Ghanem A. Sub-2 µm silica particles in chiral separation. In: Pagnola MR, Vivero JU, Marrugo AG, eds. *New Uses of Micro and Nanomaterials.* InTech; 2018.
2. Dong MW, Wysocki J. Ultraviolet detectors: Perspectives, principles, and practices. Chromatographyonline.com. https://www. chromatographyonline.com/view/ultraviolet-detectors-perspectivesprinciples- and-practices. Accessed May 11, 2021.
3. Cielecka-Piontek J, Zalewski P, Jelińska A, Garbacki P. UHPLC: The greening face of liquid chromatography. Chromatographia. 2013;76(21-22):1429-1437.
4. Dong MW. HPLC and UHPLC for Practicing Scientists. 2nd ed. Standards Information Network; 2019. https://play.google.com/store/ books/details?id=gPyhDwAAQBAJ.
5. HPLC DETECTORS: A BRIEF REVIEW - Instituto de .... Pdf4pro.com. https://pdf4pro.com/view/hplc-detectors-a-brief-review-instituto-de-325ed.html. Published August 8, 2018. Accessed May 11, 2021.
6. Choudhary A. Different types of HPLC detectors. Pharmaguideline. com. https://www.pharmaguideline.com/2016/01/different-types-ofhplc- detectors.html. Accessed May 11, 2021.
7. Swartz M. Seeing is believing: Detectors for HPLC.Chromatographyonline.com. https://www.chromatographyonline.com/view/seeing-believing-detectors-hplc. Accessed May 11, 2021.
8. UV/Vis Spectroscopy (UV). Perkinelmer.com. https://www.perkinelmer.com/uk/category/UV/Vis-spectroscopy-uv. Accessed May 11, 2021.
9. Mafina M-K. Major Considerations in Choosing a High-Performance UV/Vis or UV/Vis/NIR System. Perkinelmer.com. https://www. perkinelmer.com/lab-solutions/resources/docs/WHT_Major-Considerations-in-Choosing-a-High-Performance-UVVis-System.pdf. Accessed May 11, 2021.

10. Scott RPW, ed. Chapter 8 the selection of the appropriate detector. In: Liquid Chromatography Detectors. Vol 33. Elsevier; 1986:235-261.
11. Analysis of Common Preservatives in Personal Care Products by HPLC with UV Detection. PerkinElmer. https://www.perkinelmer.com/uk/libraries/APP-Analysis-of-Common-Preservatives-in-PCPs. Accessed; 11/06/2021.
12. RUGGED, ROBUST, AND RELIABLE PERFORMANCE EVERY DAY. Perkinelmer.com. https://www.perkinelmer.com/lab-solutions/resources/ docs/BRO_012040_01_Altus_HPLC.pdf. Accessed May 11, 2021.
13. Analysis of 16 Cannabinoids Using the PerkinElmer LC 300 HPLC System with PDA Detection. PerkinElmer. https://www.perkinelmer. com/uk/libraries/app_analysis-of-16-cannabinoids. Accessed; 11/06/2021.
14. Analysis of Phytoestrogen Isoflavones in Dietary Supplements by HPLC/UV. PerkinElmer. https://www.perkinelmer.com/uk/libraries/ app_isoflavones-in-dietarysupplements. Accessed; 11/06/2021.
15. Liquid Chromatography. Ufl.edu. Accessed May 11, 2021. https://cao.chem.ufl.edu/wp-content/uploads/sites/22/2015/01/Lecture26-2015.pdf.
16. Series 200 Fluorescence Detector- The sensitivity you need from a name you respect. Perkinelmer.com. Accessed May 11, 2021. https://www.perkinelmer.com/Content/relatedmaterials/productnotes/prd_series200fluorescencedetector.pdf.
17. PAHs in Surface Water by PDA and Fluorescence Detection. PerkinElmer. https://www.perkinelmer.com/uk/libraries/APP_PAHs-in-Surface-Water-012102_01. Accessed; 11/06/2021.
18. Dolan JW. Avoiding refractive index detector problems. Chromatographyonline.com. Accessed May 11, 2021. https://www.chromatographyonline.com/view/avoiding-refractive-index-detector-problems-0.
19. Biocompare.com. Accessed May 11, 2021. https://www.biocompare.com/Lab-Equipment/13033-HPLC-Refractive-Index-Detector-HPLC-RI-Detector/.
20. Analysis of Sugars in Maple Syrup by HILIC with Refractive Index Detection. PerkinElmer. https://www.perkinelmer.com/uk/libraries/app_sugars-in-maple-syrup. Accessed; 11/06/2021.
21. Taylor T. HPLC detector selection — what, where, when, and how. Chromatographyonline.com. Accessed May 11, 2021. https://www.chromatographyonline.com/view/hplc-detector-selection-what-where-when-and-how-0.
22. Chromatography Simplified. PerkinElmer. https://www.perkinelmer.com/uk/category/liquid-chromatography-hplc-uhplc-instruments. Accessed; 11/06/2021.

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