HPLC DETECTORS: A BRIEF REVIEW

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☐ *In an HPLC system the detector is the component responsible for turning a physical or chemical attribute into a measureable signal corresponding to concentration or identity.[1,2] In the early days of HPLC, detection was often carried out by collecting fractions and analyzing them off-line. It wasn't until the 1940's and 1950's that the first online detectors for HPLC were introduced.[3,4] Certainly an improvement over off-line approaches, sensitivity was an issue. The search for more sensitive universal detectors for HPLC led researchers to adapt GC detectors for use in HPLC,[5–8] but the removal of the HPLC mobile phase originally limited applicability. However, in the 1960s the first ultraviolet detector for HPLC was introduced,[9] and subsequent improvements in design led to better sensitivity[10] and improvements such as variable wavelength and diode array detectors. While a truly universal HPLC detector with GC/FID levels of sensitivity is still elusive, many different types of detectors have been developed that have been very successful for a wide variety of general or specific HPLC applications. To assist in choosing the proper HPLC detector for a given application, this review describes the principles and attributes of many of the common HPLC detectors in use today, and compares and contrasts the advantages and disadvantages of the various detectors discussed where appropriate. For additional detail on the HPLC detectors addressed here, the reader is encouraged to consult the references in each section along with additional general reviews.[11,12]*

Keywords chromatography, detectors, review, selection, type

INTRODUCTION

Detectors for HPLC are designed to take advantage of some physical or chemical attribute of either the solute or mobile phase in the chromatographic process in one of four ways:[2]

- A bulk property or differential measurement
- Analyte specific properties
- Mobile phase modification
- Hyphenated techniques

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Bulk property detectors are the most universal detectors for HPLC as they measure properties common to all analytes by measuring differences in the mobile phase with and without the sample. One of the most common bulk property detectors is the refractive index detector. Given the universal nature of bulk property detectors, they respond to all analytes, placing more emphasis on the selectivity of the chromatographic column. They are, however, inherently somewhat limited in sensitivity since they are the chromatographic equivalent of determining the weight of a sailor by weighing the battleship before and after the sailor departs for shore leave.

Analyte specific property detectors respond to a characteristic that is unique to an analyte. The UV detector is the most common example of an analyte specific property detector, responding to analytes that absorb UV light at a particular wavelength. UV detectors are usually thought of as somewhat specific, responding only to compounds with chromophores, but at low UV wavelengths (<210 nm), where just about every organic compounds absorb, UV detectors are actually somewhat universal. Other analyte specific detectors include fluorescence, conductivity, and electrochemical.

Mobile phase modification detectors change the mobile phase post-column to induce a change in the properties of the analyte, for example, by creating particles suspended in a gas phase. Evaporative light scattering and corona discharge detectors fit into this category. Pre- or post-column derivatization of the analyte is also sometimes considered to fit into this category, but is outside the scope of this review and will not be discussed here.[13]

Hyphenated techniques refer to the coupling of a separate independent analytical technology to an HPLC system. The most common is mass spectrometry (LC-MS), and technologies such as infrared spectrometry (LC-IR) and nuclear magnetic resonance (LC-NMR) have also been used. While it can be argued that MS is seeing more and more use as a routine HPLC detector, good reviews of its use as well as other hyphenated techniques are available and can be consulted for more information.[14–16]

There are many characteristics to consider when choosing a detector, and Table 1 lists some of them. Since no one detector has all of these characteristics, over time a multitude of detectors have been designed, produced, and sold to answer one particular challenge or another. Ease of use, predictability, and reproducibility are all very important characteristics; however, recently there is an increased emphasis on the flow cell contribution to band broadening and faster detector responses. This emphasis is due to new, low dispersion ultra high performance liquid chromatography (UHPLC) systems designed to take full advantage of sub-two μm particle size column packings.[17,18] A common rule of thumb says that for good peak integration, a minimum of 20 points should be

TABLE 1 Desired Detector Characteristics

- High sensitivity and reproducible, predictable response
- Respond to all solutes, or have predictable specificity
- Wide linear dynamic range; Response that increases linearly with the amount of solute
- Response unaffected by changes in temperature and mobile phase flow
- Respond independently of the mobile phase
- Not contribute to extra-column band broadening
- Reliable and convenient to use
- Nondestructive of the solute
- Provide qualitative and quantitative information on the detected peak
- Fast response

collected across the peak. With the chromatographic efficiency of sub-two µm particle size column packings often resulting in peaks that are less than a second wide, faster data rates, as illustrated in Figure 1 are required to maintain good integration, sensitivity, and resolution. Narrower peaks will also require smaller volume detector flow cells to maintain peak concentration, and this lower dispersion should not come at a loss of signal. Many detectors, including UV, PDA, FL, ELSD, and CAD are commercially available in both HPLC and UHPLC version depending upon the application. Figure 2 further illustrates the effect of sampling rate on sensitivity and resolution. Some additional considerations to be made when selecting a detector are summarized in Table 2. Most of the considerations in Table 2 are relatively straight forward, however the use of complimentary or orthogonal detectors used in combinations (series or parallel, depending upon backpressure limitations and/or whether or not they are destructive) should be noted as it is gaining in popularity, particularly in drug discovery or other screening-type applications. The advantages of using orthogonal detectors is shown in Figure 3, where the differences in response for a UV
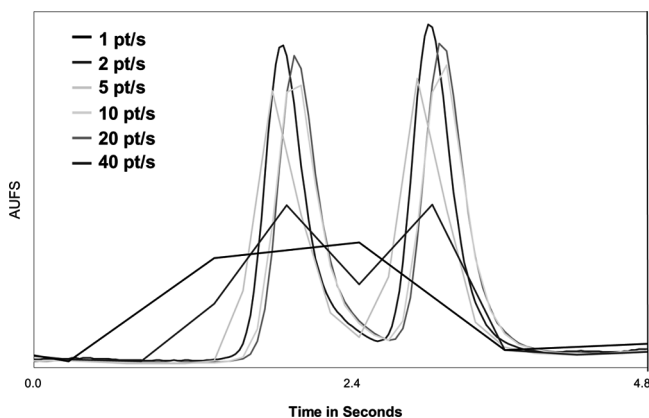

FIGURE 1 Affect of data rate on peak shape for narrow UHPLC peaks. (Figure used with permission from Advanstar Communications/LCGC Magazine.)
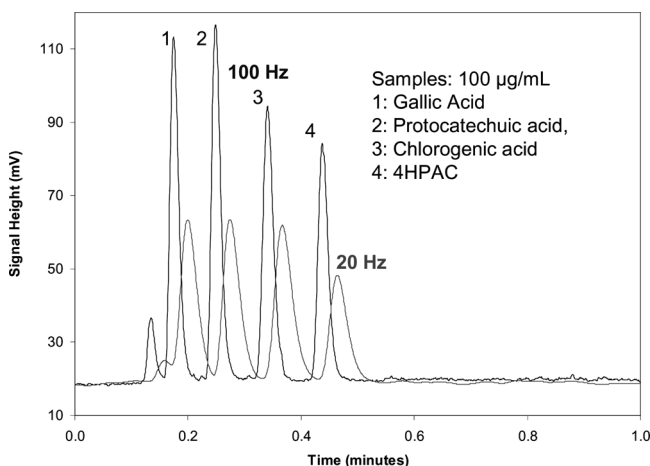
FIGURE 2 Affect of data rate on sensitivity and resolution using CAD 20 Hz versus 100 Hz. Reversed phase C_{18} separation (2.1 × 50 mm), 1.2 mL/min. at 40°C. Mobile phase: 10% ACN, 50 mM ammonium formate, adjust to pH 3 with formic acid. (Figure courtesy of ESA, Inc., Chelmsford, MA.)

(214 nm), evaporative light scattering detector (ELSD), and MS (positive electrospray ionization) can be seen for some model analytes. Table 3 lists some properties of the common HPLC detectors in use today, and a summary of some of their key attributes is presented in Table 4.

UV-VISIBLE DETECTORS

The UV-visible absorbance detector is the most common HPLC detector in use today since many compounds of interest absorb in the UV (or visible) region (from 190–600 nm). Sample concentration, output as absorbance, is determined by the fraction of light transmitted through the detector cell by Beer's Law:

$$A = \log(I_0/I) = \epsilon bc$$

where A is absorbance, I_0 is the incident light intensity, I is the intensity of the transmitted light, ε is the molar extinction coefficient of the sample, b is

TABLE 2 Example Detector Selection Criteria

- Chemical nature of analytes and potential interferences
- Limit of detection/quantitation required
- Availability and/or cost of detector
- Compatibility with HPLC/UHPLC system
- The number of different assays to be performed
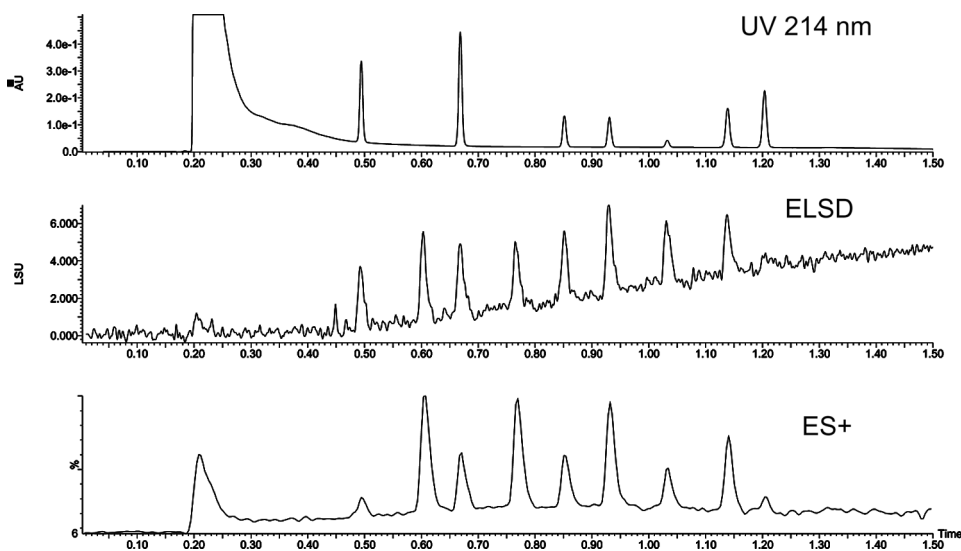- Complimentary and Orthogonal Detection

FIGURE 3 Example of orthogonal detection in UHPLC. The three detectors (UV @ 214 nm, ELSD, and MS with positive electrospray) provide complimentary information for the test mix sample. (Figure used with permission from Advanstar Communications/LCGC Magazine.)

the path length of the cell in cm, and c is the molar sample concentration. UV absorbance occurs as a result of the transition of electrons from $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$, or $n \rightarrow \sigma^*$ molecular orbitals; most aromatic compounds absorb strongly at or below 260 nm, compounds with one or more double bonds (e.g., carbonyls, olefins) at ∼215 nm, and aliphatic compounds ∼205 nm. Table 5 lists some of the more common UV chromophores and their molar extinction coefficients.[19,20] Mobile phase solvent and buffer selection is also important for optimum UV sensitivity and linearity; UV cutoffs (the wavelength at which the solvent absorbs) become particularly significant at low wavelengths.

There are three different types of UV detectors: fixed wavelength detectors that rely on distinct wavelengths, and variable and photodiode array detectors that rely on one or more wavelengths generated from a broad spectrum lamp. Fixed wavelength detectors, the backbone of early HPLC systems, are cheap and simple, but are in limited use today. The most common fixed wavelength detectors use the 254 nm output from a low pressure mercury lamp, the reason many variable wavelength and photodiode array applications today still use this wavelength out of sheer habit.

Variable wavelength detectors can be tuned to operate at the absorbance maximum of an analyte or at a wavelength that provides more selectivity. They can also be programmed to change wavelengths during a chromatographic run to compensate for response of different analytes. In a variable wavelength detector, light from a broad spectrum (for UV

TABLE 3 Common HPLC Detector Properties

	RI	Conductivity	Light Scattering	Corona Discharge	UV/VIS/PDA	FL	Radioactivity	EC
Range of Applications	Universal	Selective	Universal	Universal	Selective; Universal at low λ	Very Selective	Selective	Very Selective
Minimal Detectable Quantity	μg	High ng	High ng	Low ng	ng	pg	pg	fg – pg
Linear Range	10^3	10^4	10^3	10^4	10^5	10^3–10^4	10^3–10^4	10^5

1135

TABLE 4 Common HPLC Detector Attributes

Detector	Key Attributes	Limitations
UV/Vis/PDA	Most widely used and accepted; Near "universal" at low UV; Gradient compatible Qualitative and Quantitative; PDA peak purity/homogeneity, spectral library searches/ID, contour maps and 3D spectral display; Nondestructive Cost; Very Reliable; Easy to use	Must have a chromophore; Solvents must be transparent; Widely varying response for different solutes
Light Scattering	Detects most non volatile analytes; Works well with gradient HPLC; Better sensitivity than RI detection	Requires the use of volatile buffers, optimization; Limited dynamic range; Reproducibility of methods
Corona discharge	Highest sensitivity of "universal" type detector; Wide dynamic range; Detects any non volatile or semi-volatile; Consistent response; Ease of use	Requires the use of volatile buffers
FL	Very selective and sensitive; Works well with gradients	Not all compounds fluoresce; Often requires derivative formation; Quenching; Cost for performance
Radioactivity	Gradient compatible; can determine distribution and mass balance for drug metabolite studies, wide response range	Large flow cell volumes increase peak broadening and decrease resolution
EC	Very selective and sensitive; Modern ECs are reliable and easy to use	Mobile phase must be conductive; susceptible to background noise and electrode fouling; only applicable to compounds that can be oxidized or reduced
Conductivity	Detector of choice for ion chromatography-inorganic ions and organic acids; Very selective; Low cost	Requires suppression of mobile phase background conductivity; Not all compounds are detected; Requires special HPLC systems and columns
RI	Original detector for HPLC in many methods; Excellent versatility/Universal detection; Solvent compatibility; Nondestructive; Cost; reliable and easy to operate	Sensitivity; Gradient incompatible; Stability (Temperature and Flow)

deuterium is common, tungsten for visible) lamp is directed through a slit to a diffraction grating that spreads the light out into its constituent wavelengths. The grating is then rotated to direct a single wavelength of light through a slit, through the detector cell, to a photodiode. An example schematic for a variable wavelength detector is shown in Figure 4.

Photodiode array detectors (PDA) have an optical path similar to variable wavelength detectors except the light passes through the flow cell prior to hitting the grating, allowing it to spread the spectrum across an array of photodiodes, as illustrated in Figure 5. Z-path or tapered detector

TABLE 5 Common UV Chromophores and Molar Extinction Coefficients

Compound Type	Chromophore	λ_{max} (nm)	Molar Extinction Coefficient
Aldehyde	$-CHO$	210	1,500
		280–300	11–18
Amine	$-NH_2$	195	—
Azo	$-N{=}N$	285–400	3–25
Bromide	$-Br$	280	300
Carboxyl	$-COOH$	200–210	50–70
		280	16
Bisulfide	$-S-S$	194	5,500
		255	400
Ester	$-COOR$	205	50
Ether	$-O-$	185	1000
Iodide	$-I-$	260	1000
Ketone	$C{=}O$	195	1,000
		270–285	15–30
Nitrate	$-ONO_2$	270	12
Nitrite	$-ONO$	220–230	1000–2000
		300–400	10
Nitro	$-NO_2$	210	Strong
Oxime	$-NOH$	190	5000
Phenol	C_6H_5-OH	270	1,450
Unsaturation, Conjugated	$-(C{=}C)_3-$	260	35,000
	$-(C{=}C)_4-$	300	52,000
	$-(C{=}C)_5-$	330	118,000
Unsaturation, Aliphatic	$-(C{=}C)_2-$	210–230	21,000
Benzene	C_6H_6	184	46,700
		202	6,900
		255	170
Diphenyl	$C_{12}H_{10}$	246	20,000
Miscellaneous	$C{=}C-C{=}N$	220	23,000
	$C{=}C{=}C{=}O$	210–250	10,000–20,000
	$C{=}C-NO_2$	229	9,500

Data extracted from references [19] and [20].
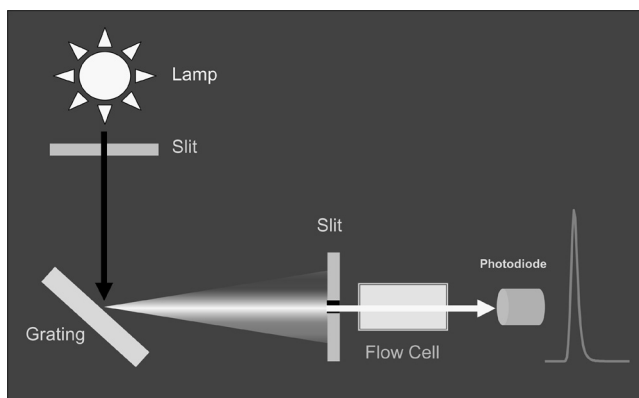
FIGURE 4 Variable wavelength UV detector schematic.

cells designs are commonly used in most UV detectors for HPLC. Using a
4.6×100 mm column ($3 \mu m$ particle size) detector cell volumes of $8-10 \mu L$
are required (e.g., 1 mm diameter, 10 mm path length). An $8 \mu L$ flow cell
volume may lead to unacceptable extra-column band broadening using
smaller column diameters and particle sizes; however, reducing the path
length to decrease extra-column volume will decrease the signal. In
addition, early PDA detectors were not as sensitive as single wavelength
UV, but the gap has been closing in recent years thanks to new detector cell
technology of the type in use in some detectors today, as illustrated in
Figure 6. This new detector cell type consists of a light guided flow cell
equivalent to an optical fiber. Light is efficiently transferred down the flow
cell in an internal reflectance mode that still maintains a 10 mm flow cell
path length with a volume of only 500 nL; extending the path length while
maintaining low dispersion. An example of the sensitivity and the dynamic
range that is possible with a PDA detector is shown in Figure 7 for an active
pharmaceutical ingredient (API) impurity at the 0.01% level. Light guided
flow cells are particularly useful in maintaining low dispersion in UHPLC
systems.

PDA's extend the utility of UV detection by provide spectra of eluting
peaks that can be used to aid in peak identification, and to monitor for
co-elutions (peak homogeneity or purity), helpful during method
development. They can also serve as a multi-wavelength UV/VIS detector.
The spectra collected at the chromatographic peak apex can be used to
create a library that can in turn be used to compare subsequent spectra
for identification purposes, and spectra collected across the peak at each
data point can be compared to evaluate peak homogeneity or purity. The
added spectral resolution of modern PDA detectors coupled with chroma-
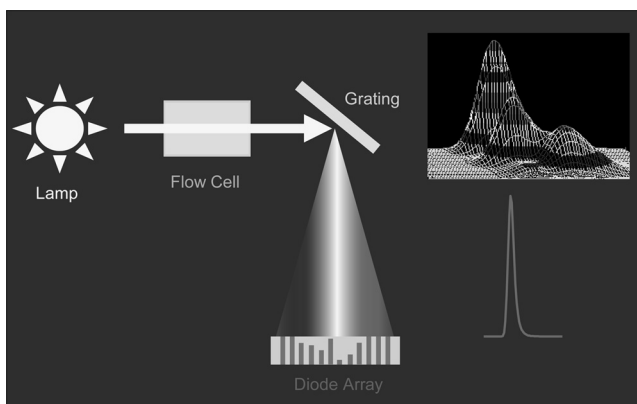tography data system (CDS) software algorithms can quickly compare fine

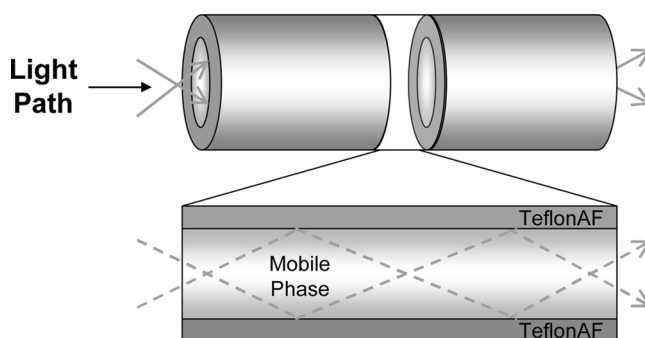
FIGURE 5 PDA detector schematic.

FIGURE 6 Schematic representation of a light guided detector flow cell. (Figure used with permission from Advanstar Communications/LCGC Magazine.)

differences in the spectra not clearly visible to the eye. Some comparisons are done by a simple direct point-to-point comparison of spectra; whereas, in other comparisons, complex vector analysis in multi-dimensional space is performed to look at spectral fine structure. In the latter case, the magnitude of the angles between the calculated vectors indicates how different the spectra are, as illustrated in Figure 7. For example, in a library match, if the angle of the vectors between the library spectra and the unknown spectra are zero, there is a high likelihood the two compounds giving rise to the spectra are the same. To determine peak purity, spectral comparisons are made against a threshold value, which takes into account noise. In
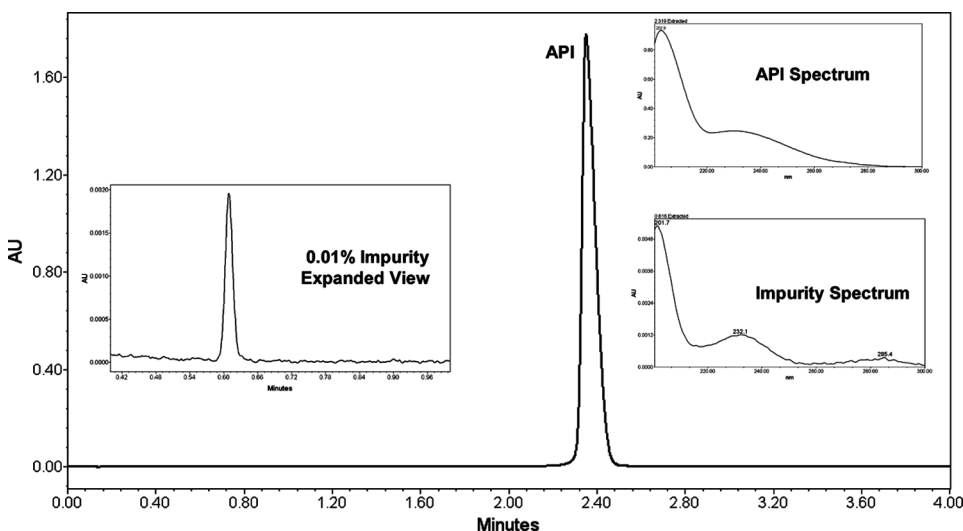

FIGURE 7 PDA detector response of the chromatographic analysis of an API and impurity at the 0.01% level. Insets show an expanded view of the chromatogram in the region where the impurity elutes, and the PDA derived UV spectra for both the API and the impurity. (Figure used with permission from Advanstar Communications/LCGC Magazine.).
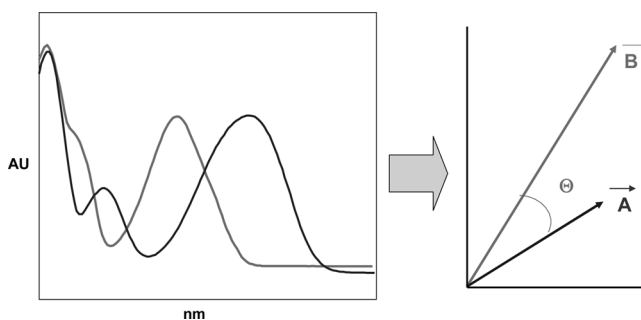
FIGURE 8 Converting spectra to vectors for mathematically enhanced spectral analysis and spectral contrast evaluation. To compare the two vectors A and B, angle Θ is calculated after the spectra are reduced point by point to vectors. If vectors A and B are totally different, $\Theta = 90°$. If A and B totally identical, $\Theta = 0°$. (Figure used with permission from Advanstar Communications/LCGC Magazine.)

order for spectral comparisons to work the compound(s) must have some UV absorbance, and there must be some degree of spectral and chromatographic resolution. Spectra will also be changed if the organic concentration or pH is altered, for example, during method development. The changes in spectra resulting from mobile phase differences often result in a shifting of the spectra, affecting the quality or the "fit" of the match, but not necessarily the information obtained. In the examples shown here, the absolute values of the calculated angles will change, but not relative to each other when comparing spectra collected under different mobile phase conditions. Figure 8 shows how CDS software peak purity plots can be used to monitor chromatographic peaks for co-elution.

FLUORESCENCE DETECTORS

Fluorescence detectors (FL) measure the optical emission of light by solute molecules after they have been excited at a higher energy wavelength and can be very sensitive for compounds that have native fluorescence or that can be made to fluoresce through derivatization.[13] Schematically, they resemble Figure 4, except that the grating is replaced by a filter or monochromator at a right angle to the incident light to simplify the optics and reduce background noise. The light source is usually a broad spectrum deuterium or xenon flash lamp. The excitation wavelength (often close to the UV λ_{max}) is selected by a filter or monochromator between the lamp and the flow cell, always at a higher energy (lower wavelength) than the emission wavelength. Laser-induced fluorescence (LIF) detectors using lasers as the excitation source are sometimes used in micro- or capillary-LC systems, where the higher energy of the laser provides better sensitivity in the small diameter flow cells necessary to limit dispersion.

FL detectors can be as much as 100 times more sensitive than a UV detector, making them particularly useful for trace analyses, or in sample limited or low concentration sample situations. While the dynamic range (the range over which a change in sample concentration produces a change in the detector output) of FL detectors is relatively large (Table 1), the linear dynamic range is often smaller for many analytes. Care should be taken to properly select and care for mobile phase components as some buffers or solvents can cause background fluorescence, and solvents that are not properly degassed can lead to quenching, both affecting sensitivity

ELECTROCHEMICAL DETECTORS

For compounds that can be oxidized or reduced the electrochemical (EC) detector is one of the most sensitive and selective HPLC detectors available.[21,22] EC detectors require the use of electrically conductive HPLC mobile phases (buffers suffice) and, when properly used and maintained, are the standard bearer when it comes to response levels for the HPLC analysis of compounds such as catecholamines and neurotransmitters. EC detectors for HPLC usually contain three separate electrodes; a working, a counter (auxiliary), and a reference electrode. Common electrode materials are carbon, gold, silver, or platinum. A fixed potential difference is applied between the working electrode and the reference electrode to drive an electrochemical reaction at the working electrode's surface. Current produced from the electrochemical reaction as compounds are oxidized or reduced at the working electrode is balanced by a current flowing in the opposite direction at the counter electrode. The EC detector response output is the amplified current resulting from the electrochemical reaction at the working electrode.

Amperometric EC detectors use disk type thin-layer electrodes operated at a constant potential. They exhibit low conversion efficiencies due to diffusion limitations and are only capable of measuring 5–10% of an electrochemically reactive analytes; however, in spite of the low conversion efficiencies, they are still quite sensitive. Amperometric EC detectors can be run in a pulsed mode (referred to as pulsed amperometric detection, or PAD) where a cycled potential is used to clean and restore the electrode surfaces several times a second, prior to measuring the signal output. PAD's are the detector of choice when sensitive carbohydrate measurements are required.

Coulametric EC detectors overcome the limited conversion efficiencies of the amperometric detectors by using a flow-through "frit" type porous graphite working electrode. They exhibit near 100% conversion efficiencies for electrochemically reactive species. Because of the higher conversion
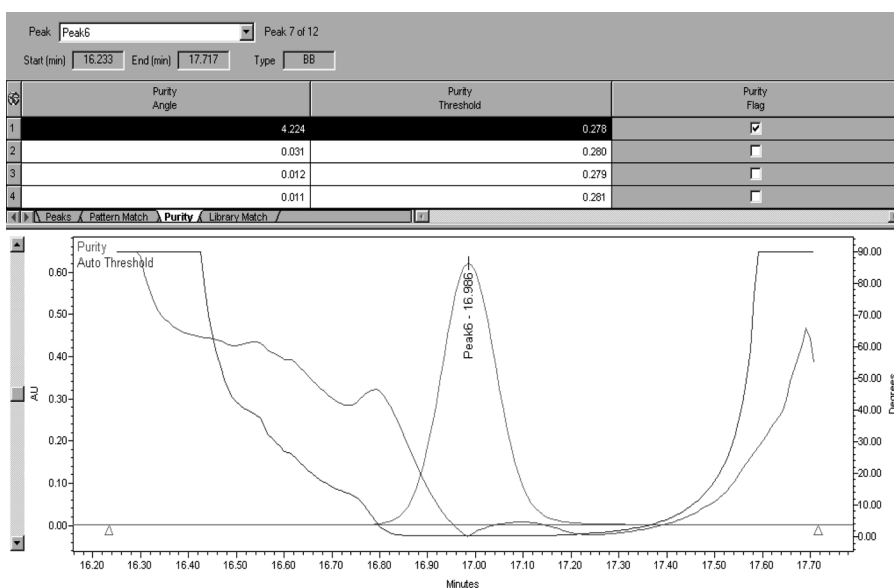
FIGURE 9 Example peak purity plot from for mathematically enhanced spectral analysis and spectral contrast. By plotting peak purity angles versus threshold angles for spectra collected across the peak, plots such as these can be used to determine co-elutions indicated when the purity angle is greater than the threshold angle. In this example from a simple reversed phase HPLC separation, a co-eluting impurity is indicated in the front edge of the peak. (Figure used with permission from Advanstar Communications/LCGC Magazine.)

efficiency, coulametric EC detectors are more sensitive and robust than their amperometric counterparts and still have the flexibility to operate in amperometric, pulsed amperometric, and cyclic voltametry modes in addition to coulametric.

The USP recently added a method using high-performance anion-exchange with PAD (HPAE-PAD) to identify and quantitate the level of organic impurities in heparin due to recent health concerns.[23–26] The organic impurities section of the new heparin USP monograph relies on hydrolyzing the polysaccharide and determining the relative amounts of galactosamine and glucosamine in the sample digests by analysis on an anion exchange column with electrochemical detection. Heparin is composed of glucosamine and uronic acid, and acid hydrolysis of heparin samples releases the glucosamine, which is readily determined with high sensitivity by pulsed amperometric electrochemical detection (PAD). In comparison, over-sulfated chondroitin sulfate (OSCS-the adulterant) is composed of galactosamine and uronic acid. In these compounds, acid hydrolysis releases galactosamine, also easily determined by electrochemical detection. The USP method measures the ratio of galactosamine/glucosamine as an indication of the heparin purity and to identify heparin
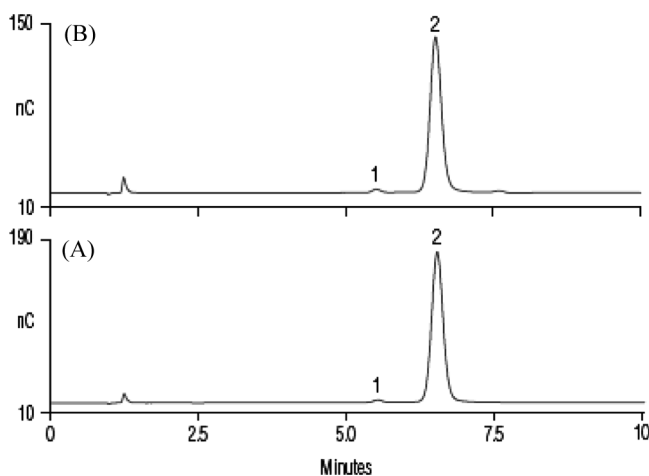
FIGURE 10 Analysis of galactosamine impurities in heparin according to the USP monograph in reference 23 (see also reference 25). Separation A is from a standard solution; B from a digested spiked heparin sample. Peak 1 is galactosamine (1% level w/w), peak 2 is glucosamine. Additional method details can be found in references 27 and 30. (Figure used with permission from Advanstar Communications/LCGC Magazine.)

samples that may be contaminated or adulterated with chondroitin sulfate compounds. An example of the separation is shown in Figure 10. The combination of the anion exchange separation and the sensitivity of PAD allows for the detection of galactosamine at levels as low as 0.4%.[25]

RADIOACTIVITY DETECTORS

Radioactivity detectors (sometimes referred to as radiometric or radio-flow detectors) are used to measure radioactive analytes as they elute from the HPLC column. Most radioactivity detectors are based on liquid scintillation technology to detect phosphors caused by the radioactive nuclides, such as low-energy β-emitters (e.g., ^{35}S, ^{14}C. ^{3}H, and ^{32}P) or stronger α-, β-, and γ-emitters (e.g., ^{131}I, ^{210}Po, and ^{125}Sb); tritium and ^{14}C being the most common. A liquid scintillator can be added post column (called homogeneous operation) or the flow cell can be packed with beads of a permanent solid state scintillator (heterogeneous operation). The radioactivity detector can be very sensitive and is extremely useful for the detection of radiolabeled compounds in toxicological, metabolism, or degradation studies. Large flow cell volumes are typically used in radioactivity detectors to increase analyte residence time, which increases the number of radioactive decays that can be detected. Peak tailing and broadening caused by the larger cell volume can be minimized by using larger volume columns assuming that sufficient sample is available for larger mass injections to compensate for

on column dilution. The use of a large cell, column, and injection volumes generally limit radiochemical detector use in UHPLC.

CONDUCTIVITY DETECTION

The conductivity detector is a bulk property detector that measures the conductivity of the mobile phase. Conductivity detectors are the detector of choice for ion chromatography or ion exchange separations when the analyte does not have a UV chromophore.[27,28] In a conductivity detector, the resistance (or strictly the impedance) between two electrodes in the flow cell is measured. For many applications, particularly ion chromatography, where conductive buffers are required in the mobile phase, a suppressor column is used post-analytical column (before the detector) in order to reduce the background conductance of the mobile phase. An example ion chromatographic separation of organic acids using supressed conductivity detection is shown in Figure 11.

CHEMILUMINESCENT NITROGEN DETECTOR

The chemiluminescent nitrogen detector is an element specific detector where the column effluent is nebulized with oxygen and a carrier gas of argon or helium and pyrolyzed at $1050°C$.[29–31] Nitrogen containing compounds (except N_2) are oxidized to nitric oxide, which is then mixed with ozone to form nitrogen dioxide in the excited state. The nitrogen dioxide decays to the ground state with the release of a photon, which is detected by a photometer. The resultant signal is directly proportional to the amount of nitrogen in the original analyte. Because of this relationship,
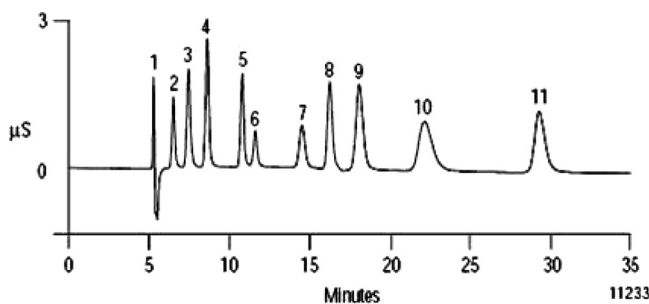

FIGURE 11 Analysis of organic acids by ion chromatography with conductivity detection. Separation was performed on an IonPac ICE AS6 using a 0.4 M perfluorobutyric acid mobile phase at 1 mL/min. Suppressed conductivity detection with 5 mM tetrabutylammonium hydroxide regenerant. Peak ID (concentration in mg/L): 1) Oxalate (5.0), 2) Tartrate (10.0), 3) Citrate (15), 4) Malate (20), 5) Formate (10.0). 6) Lactate ((10.0), 7) α-Hydroxybutyric acid (30.0), 8) Acetate (25.0), 9) Succinate (25.0), 10) Fumerate (35.0), and 11) Propionate (50.0). (Figure courtesy of Dioanex Corporation; LPN 0660.)

it is possible to calibrate the response with any compound with known nitrogen content and quantitate the nitrogen content of unknown analytes. Care must be taken in choosing nitrogen free mobile phases components and additives (e.g., no acetonitrile!).

CHIRAL DETECTORS

Many compounds, particularly drugs, exist in enantiomeric forms that can possess significantly different pharmacological properties, and chromatographic separation of enantiomers can be complimented by the use of detectors capable of responding to the different chiral forms. Chiral detectors in flow cell form essentially mimic their bench top counterparts; polarimeters (PL), optical rotary dispersion (ORD), and circular dichroism detectors (CD).[32] Polarimeters measure the degree of rotation of polarized light as it passes through the sample. The amount of rotation is dependant upon both the concentration and molecular structure of the analyte. ORD detectors operate quite similar to polarimeters but at lower wavelengths. CD detectors measure the difference in absorption of right and left circularly polarized light as an analyte flows through the detector cell. Chromophores with absorption in the 200–420 nm range yield the best responses. CD detectors have typically proven to be the most sensitive. One additional chiral detector note: detector response can yield both positive and negative peaks relative to a normal baseline, a requirement that should be kept in mind when considering CDS software.

REFRACTIVE INDEX DETECTION

The refractive index (RI) detector is a universal bulk property detector, and is the original, oldest LC detector. RI detectors measure the difference in optical refractive index between mobile phase and the sample; no chromophore on the solute molecule is required. For this reason, RI detection has been used very successfully for the analysis of sugars, triglycerides, and organic acids. The most common RI detector design is the deflection refractometer where the light from a tungsten source lamp is directed through a pair of wedge-shaped flow cells, (reference and sample). The reference cell contains trapped or static mobile phase and the column effluent is sent through the sample cell. As the 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. Modern RI detectors use thermostatted flow cells due to the susceptibility of RI measurements to temperature fluctuations. Once a chromatographic mainstay, and still used in many GPC applications, refractive index (RI) detection is

being replaced in many applications by light scattering or corona discharge detectors due to its limited sensitivity and gradient incompatibility.

LIGHT SCATTERING DETECTORS

Recent improvements in the ability to efficiently nebulize an HPLC column effluent has lead to increased utility of light scattering detectors. The most popular detector of this type is the evaporative light scattering detector (ELSD). The ELSD works on the principle of evaporation (nebulization) of the mobile phase followed by measurement of the light scattered by the resulting particles. The column effluent is nebulized in a stream of nitrogen or air carrier gas in a heated drift tube and any nonvolatile particles are left suspended in the gas stream. Light scattered by the particles is detected by a photocell mounted at an angle to the incident light beam. Carrier gas flow rate and drift tube temperature must be adjusted for whatever mobile phase is used. Detector response is related to the absolute quantity of analyte present, and while decreased sensitivity will be obtained for volatile analytes, unlike the UV detector, no chromophores are required and it has orders of magnitude more response than the RI detector. ELSD also has the advantage over RI detection in that the response is independent of the solvent, so it can be used with gradients, and is not sensitive to temperature or flow rate fluctuations. Mobile phases of course must be volatile, similar to those used for MS detection. Linearity can be limited in some applications, but is certainly quantitative over a wide enough range if properly calibrated. Recent applications of the ELSD have also been extended to UHPLC; an example separation comparing low wavelength UV to ELSD (and another example of the advantages of orthogonal detection) for the separation of some antibiotics are presented in Figure 12.

The condensation nucleation light scattering detector (CNLSD) is another form of light scattering detector that is used in the same type of applications suited for the ELSD. In CNLSD, following evaporation of the mobile phase, a saturated stream of solvent is added to the particles in the carrier gas. The particles form condensation nuclei and the solvent condenses onto the particles, increasing their size to where they are more easily detected in the light path.[32] Due to the increase particle size, the CNLSD can be 10–100-fold more sensitive than ELSD, with a wider linear range.

Laser light scattering detectors (LLSD), also called multi-angle light scattering (MALS) refer to light scattering detectors that make measurements in solution as opposed to particles suspended in a gas. LLSDs use a laser light source, and measure scattered light from the sample as it moves through the detector flow cell. The scattered light is measured at multiple (e.g., 3–18) angles, and using the proper mathematical transformations
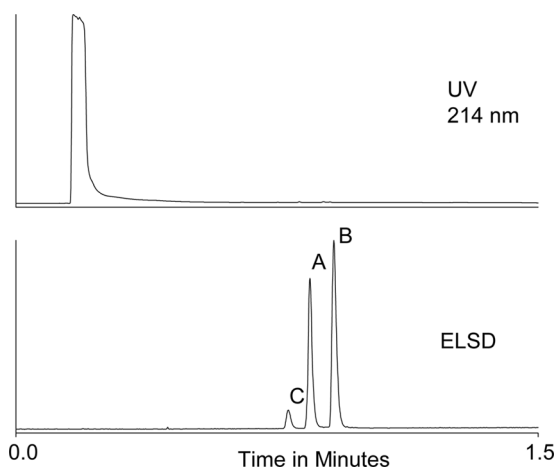
FIGURE 12 Comparison of UV and ELSD Response for a UHPLC Separation of Antibiotics Erythromycins A, B and C. Reversed phase UHPLC separation. (Figure used with permission from Advanstar Communications/LCGC Magazine.)

the mass of an analyte can be determined without the use of reference standards. LLSD is particularly useful in size exclusion chromatography for the determination of the molecular weights of polymers or biological molecules.

CORONA DISCHARGE DETECTION

Corona charged aerosol detection (CAD), sometimes referred to as corona discharge detection (CDD) is a unique technology gaining in popularity in which the HPLC column eluent is first nebulized with a nitrogen (or air) carrier gas to form droplets that are then dried to remove mobile phase, producing analyte particles. The primary stream of analyte particles is met by a secondary stream of nitrogen (or air) that is positively charged as a result of having passed a high-voltage, platinum corona wire. The charge transfers diffusionally to the opposing stream of analyte particles and is further transferred to a collector where it is measured by a highly sensitive electrometer, generating a signal in direct proportion to the quantity of analyte present. A simplified schematic of how the CAD works is illustrated in Figure 13.

Because the entire process involves particles and direct measurement of charge, CAD is highly sensitive, provides a consistent response, and has a broad dynamic range, which offers some real advantages, particularly when analyzing compounds lacking UV chromophores. Often compared to other universal-type HPLC detectors, like RI and ELSD, CAD has been shown to be much easier to use, and unlike RI, can accommodate gradients. In addition, CAD response is not dependent upon the chemical characteristics of the compounds of interest, but on the initial mass concentration of analyte in
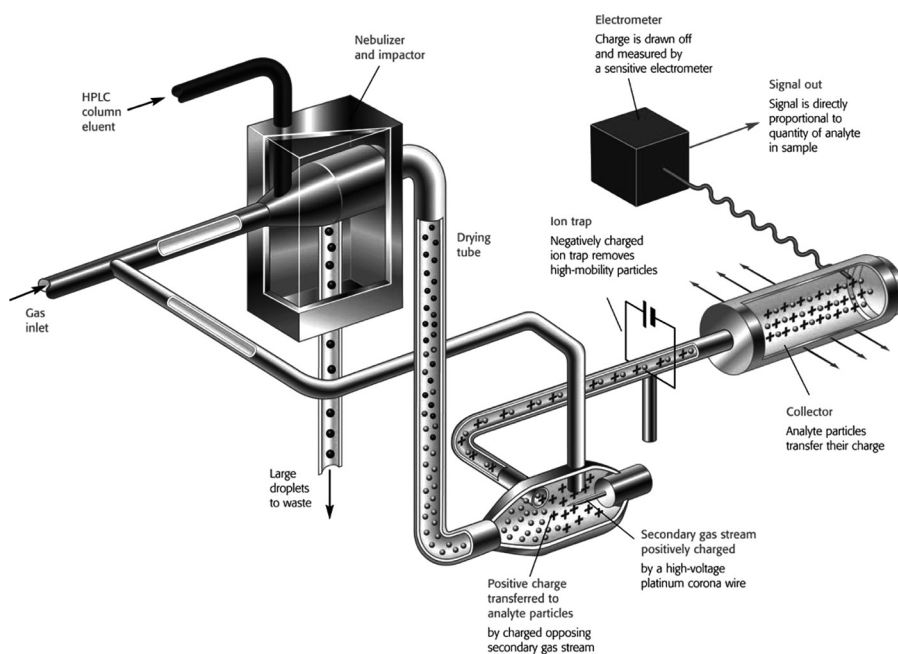
FIGURE 13 A simplified schematic of a corona charged aerosol detector. (Figure Courtesy of ESA, Inc., Chelmsford, MA.)

the droplets formed upon nebulization, providing a much more uniform response as opposed to, for example, UV, where responses can vary dramatically according to the wavelength used and the extinction coefficient.

CAD has been used for a wide range of analyses throughout the drug development process, for example drug discovery,[34] formulations
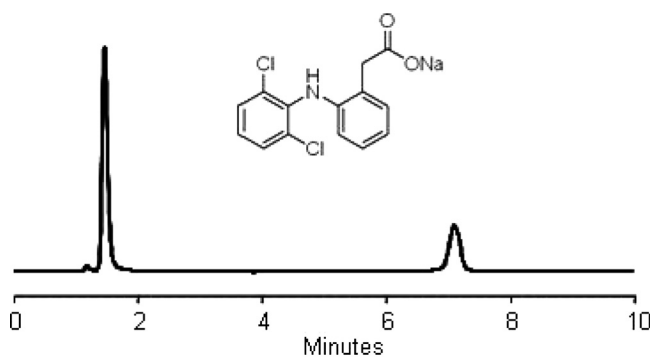

FIGURE 14 HILIC/CAD analysis of API and counterion in a single run. Conditions: A Sequant ZIC®-*p*HILIC 5 mm, 4.6 × 150 mm column (The Nest Group, Southborough, MA) operated at 30°C was used with an isocratic mobile phase of 75:25 acetonitrile/100 mM ammonium acetate (pH 7.0) at an isocratic flow rate of 1.0 mL/min, and a 10 μL injection. (Figure used with permission from Advanstar Communications/ LCGC Magazine.)
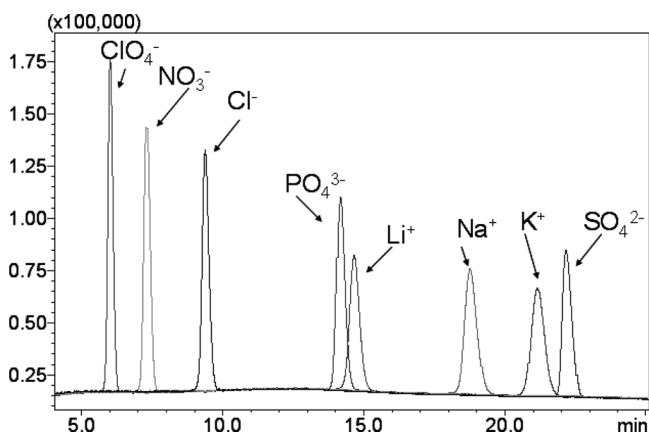
FIGURE 15 Simultaneous analysis of anions and cations using HILIC/CAD. Conditions: A Sequant ZIC®-*p*HILIC 5 mm, 4.6×150 mm column (The Nest Group, Southborough, MA) operated at 30°C was used. Gradient conditions: 20 to 70% B over 26 minutes; Mobile Phase A: 15% 100 mM Ammonium Acetate pH 4.68, 5% Methanol, 20% IPA, 60% Acetonitrile; mobile phase B: 50% 30 mM Ammonium Acetate pH 4.68, 5% Methanol, 20% IPA, 25% Acetonitrile, at a flow rate of 0.5 mL/min and a 10 μL injection. (Figure used with permission from Advanstar Communications/LCGC Magazine.)

research and development,[35] natural product isolation,[36] impurities,[37,38] cleaning validation,[39] drug substance and drug product characterization,[40,41] and stability[42] among others. In most aspects, the CAD is simple and easy to use, and can be described as a "plug and play" detector requiring little in the way of special attention, unlike an ELSD that can require carrier gas flow rate and drift tube temperature optimization and adjustments.

CAD, when used in combination with hydrophilic interaction chromatography (HILIC), offers some real advantages for hydrophilic compounds that do not have UV chromophores. Figure 14 illustrates how the HILIC/CAD method was used to analyze diclofenac and its sodium counterion in a single run. Figure 15 shows the CAD used for ion analysis and detection of ionic impurities. For compounds of this type, CAD in combination with the HILIC mode of HPLC offers some advantages over conductivity detection typically used.

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