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Vabilo na predavanje

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Specific and Nonspecific Detection in HPLC

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. 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. Ever since we differentiate between bulk property (nonspecific) and solute-specific detectors.

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 analyte. One of the most common bulk property detectors is the refractive index (RI) detector. Given the universal nature of bulk property detectors, they respond to all analytes, placing more emphasis on the selectivity of the chromatographic column. Since RI detectors are not very sensitive and not compatible with gradient elution techniques, evaporative light scattering detectors (ELSD) and charged aerosol detectors (CAD) have been introduced to overcome these problems. However, nonspecific detectors should only be used if there is no alternative.

Solute-specific detectors respond to a physico-chemical property that is unique to an analyte. The UV detector is the most common example of a solute-specific 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 compound absorbs, UV detectors are not very specific. Since the UV absorbance also differs depend on what wavelength is used, it is important to choose an appropriate wavelength based on the type of analyte. Today, the most common detectors provide a wide wavelength selection, covering both UV and VIS ranges (195 to 700 nm). Photo diode array (PDA) detectors add a third dimension (wavelength) over absorbance and retention time. This is convenient to determine the most suitable wavelength for an analyte without repeating analyses.

KEMIJSKI INŠTITUT

PDA detectors also allow the acquisition of the entire UV spectrum of a compound passing the flow cell as well as peak purity analysis. Other solute-specific detectors include fluorescence, electrochemical, and chemiluminescence.

Hyphenated techniques refer to the coupling of a separate independent analytical technology to an HPLC system. The most common is the hyphenation with mass spectrometry (LC–MS) and with ICP (LC–ICP/MS) to obtain mass-sensitive and element-specific information, respectively.

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 UHPLC systems designed to take full advantage of sub-two µm particle size column packing materials.

In this presentation, characteristics will be described that have to be considered when choosing a detector.

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Vljudno vabljeni / Kindly invited