Unlocking the Power of 3D Absorbance Data

Peak deconvolution with photodiode array (PDA) detectors using a unique software function can separate peaks that are not resolved on-column, yield better detection results, and minimize method development and analysis time.

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LCGC: What's the difference between 2D UV-vis data and 3D PDA data?
DOMANSKI: Two-dimensional UV-vis data describes the total absorbance of a sample versus time for a single chosen wavelength producing a chromatogram. This type of data only shows that a compound has some absorbance at the wavelength of observation but can't provide much more information. There is no way to tell if we are observing the compound at a wavelength of high or low absorbance or to predict what the compound might be. When we collect 3D PDA data, for each moment in time during the experiment, we can observe and record intensity across a range of wavelengths. This provides useful spectrum information that can determine maxima that yield the best limits of detection for the compounds of an assay or be used for traditional PDA data analysis such as cosine-vector peak purity.

LCGC: Beyond these uses, how does peak deconvolution unlock the full potential of PDA data?
DOMANSKI: While traditional PDA analysis tools may use a portion of the spectrum information available, peak deconvolution uses all the data and applies accepted mathematical techniques to separate peaks that are not resolved on-column. This can allow for detection of coeluting impurities in a potency assay, indicate an unexpected coeluting reaction product, or allow for characterization of a hard-to-separate degradant.

LCGC: What is the key to peak deconvolution, and how did this function get developed?
DOMANSKI: At the heart of our PDA peak deconvolution function is a sophisticated peak-fitting and spectrum-fitting algorithm that uses the multivariate curve resolution – alternating least squares (MCR-ALS) approach to determining solution sets, which is often used in benchtop spectroscopy applications such as chemometrics and observation of reaction kinetics. Because the technique can work with complex multi-component systems, it is ideal for LC-PDA data, where a single experiment may contain many peaks, each with associated

spectral data. Making the leap from accepted technique to implementation in software was done in conjunction with Eisai Co. Ltd., a leading global pharmaceutical manufacturer. Their goal was to accelerate therapeutic development by investing in technologies that would reduce analysis time and increase awareness of coelution-related issues at every stage of the process.

LCGC: How does MCR-ALS deconvolution compare to other forms of peak purity assessment?
DOMANSKI: In contrast to traditional cosine-vector peak purity or purity-angle assessment, MCR-ALS deconvolution uses the entire data set from the defined time and wavelength domain to calculate peak shape and spectrum information for each identified chromatographic feature. If users have multiple impurities, up to five peaks can be identified within a single deconvolution segment, and up to 12 segments can be set in each chromatogram. So not only can coelution be spotted, but the potential impurities can be characterized with the same tools as any other peak, including the reconstructed spectrum.

LCGC: Can the deconvoluted peaks be integrated, and are reconstructed spectra accurate?
DOMANSKI: The short answers are yes, and yes. With regards to integration, we have studied a three-component system of common pharmaceutical drugs under ultra-high-performance liquid chromatography (UHPLC) conditions that result in partial separation and elution in less than 20 seconds. The resulting chromatogram produces peaks and valleys, but none of the peaks are baseline resolved. We then compared the results of PDA peak deconvolution with the traditional integration strategy of splitting the peaks with a vertical drop from the bottom of the valley to baseline and with separate injections of each standard.

Traditional valley-to-valley definition of the unresolved peaks led to as much as 13.1% deviation from the single standard injection, whereas using the deconvolution result, peak area was much more accurately assigned to the peaks, with the greatest error versus single standard injection being 5.6%. For the same three-component system, the reconstructed spectra were compared with the observed spectra from the single standard injections. For all the reconstructed spectra, the similarity was 0.9998 or greater, indicating a near perfect match to empirical data.

LCGC: With these capabilities, where do you see PDA peak deconvolution fitting into pharma and biopharma workflows?
DOMANSKI: With its ease of implementation, this technique can potentially replace complex multi-dimensional separation or time-consuming low-flow, high-capacity columns that cannot provide rapid results in a user-friendly way. As International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) and other regulatory bodies continue to tighten requirements for impurities detection, PDA peak deconvolution is an additional arrow in the quiver of analysis with its ability to detect low-level coeluting impurities. By identifying these species at the beginning of drug development, time can be saved throughout the downstream process. For early-stage high-throughput screening, PDA peak deconvolution could allow for faster injection cycles while maintaining the quality of data.
In a medicinal chemistry setting, deconvolution could be used in UHPLC reaction monitoring to look for hidden peaks or coeluting intermediates or be applied to re-injections of purification fractions as a secondary check of purity. As the drug-development cycle continues, any in-silico testing that uses absorbance can be augmented by using PDA peak deconvolution to provide information on coeluting peaks without the need to spend additional time on LC method development. And for groups that may have experience with PDA peak deconvolution, such as analytical method research and development, the ability to use this powerful technique integrated directly into the instrument software provides unprecedented access and ease.

LCGC: Is Shimadzu planning future development of this technique?
DOMANSKI: We are developing a new generation of the PDA peak deconvolution algorithm that will improve performance in terms of sensitivity and reliability for some of the most difficult use cases. Our international team is working in cooperation with pharma users to optimize the feature for lower detection limits, even when there is minimal on-column separation.

For more information, visit:
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