Application of Multicomponent Analysis to HPLC Diode Array Detection for the Quantitation of Partially Resolved Peaks

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Introduction

Using a diode array detector allows the qualitative identification of compounds even when they are not completely resolved, but peak quantitation under these conditions is more difficult. Using multicomponent analysis (MCA), a technique that provides accurate quantitation and identification of unknown components based on known reference spectra, quantitation of diode array data is easily achieved under partial or zero resolution conditions.

Multicomponent Analysis evaluates a peak from start to end. This is necessary for accurate quantitation but has the added advantage of more thoroughly testing a peak for homogeneity than many other purity assessment techniques, which are applied only between the upslope and downslope inflection points (peak heart region). The individual peak profiles are determined by MCA over the complete wavelength range. Small peaks fused to a major peak are easily quantitated even when they are not visually detected, or integrated separately. This quantitation is done without the need to select different optimum wavelengths to distinguish the compounds.

Experimental

The determination of two herbicides, paraquat and diquat, is usually done by ion-pair HPLC in a single analysis in which they are only partially resolved (Figure 1). A typical quantitation procedure requires that data be extracted and reprocessed at two separate wavelengths (Table 1). There is a large potential for peak area assignment problems with these two compounds based on both spectral and experimental considerations.

Though the spectra for paraquat and diquat are very different (Figure 2), there is still some absorbance from each of the compounds at the recommended wavelengths for quantitation. When the two compounds are both present in a sample at very different levels, traditional peak integration and quantitation can be difficult. Non-MCA peak integration can result in only one detected peak at each quantitation wavelength with both Paraquat and Diquat contributing to the peak area. This will result in significant quantitation errors unless MCA is used. This makes quantitation very difficult.

MCA is the best method to accurately determine the amount of each compound present in the peak under these types of conditions.

Table 1 HPLC Conditions for Paraquat/Diquat Analysis

Column Conditions:

Column:	Hamilton PRP-1 5 μ, 4.1 mm x 150 mm
Column Temperature:	35.0°C
Flow Rate:	2.0 mL/min
Mobile Phase:	Ion-Pair Mobile Phase consisting of o-phosphoric acid, diethylamine, and 1-hexane sulfonic acid dissolved in water
Separation:	Isocratic

Detector Conditions:

Wavelength Range:	210 - 367 nm
Sample Rate:	1.4 Hz (Data Rate = 11 Hz, Bunch = 8)
Run Time:	5.0 min

Quantitation Wavelengths:

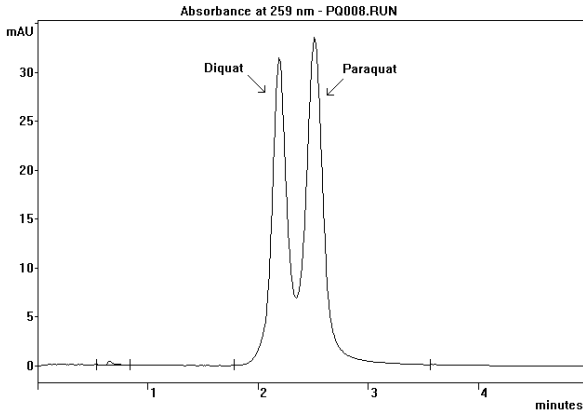|	
Paraqat:	259 nm
Diquat:	316 nm

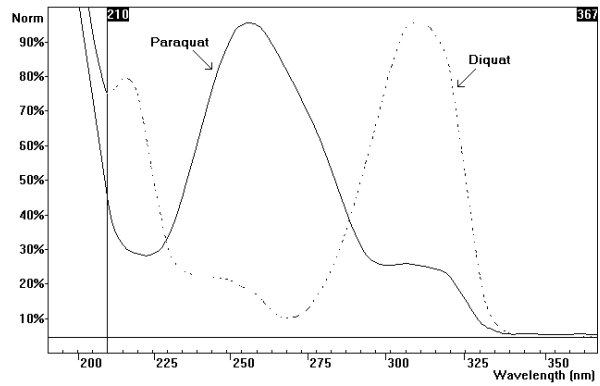Figure 1. Separation of Diquat and Paraquat

Figure 2. Diquat and Paraquat Spectra

Results

A Plot Analysis of this sample separation at very different levels is shown in Figure 3. Paraquat is at its optimum wavelength and present at 10x the concentration level of Diquat. This figure is part of the standard MCA report which includes a plot analysis (top) and a corresponding error analysis (bottom). Figure 4 shows an MCA Plot Analysis of the same sample at the optimum wavelength for Diquat. At these wavelengths the smaller component causes quantitation errors in the major component. Since there is nothing that exceeds the noise threshold (Figure 3), the peak contains only the Paraquat and Diquat.

Plot Analysis at 259 nm

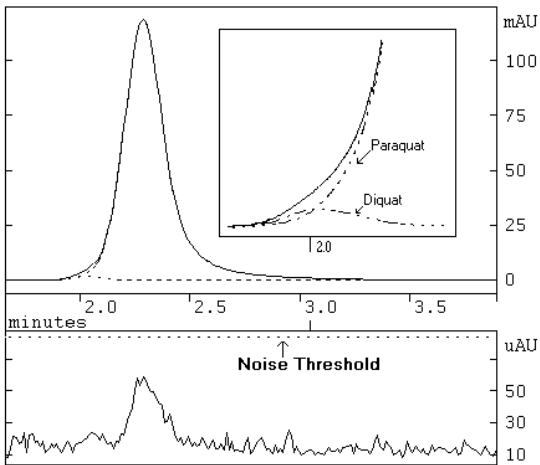| Library Index	t_R (min)	Height mAU	Width (sec)	Resolution
#2	2.015	1.84	8.8	—
#1	2.286	118.40	12.3	0.90

Figure 3. Plot Analysis of Paraquat and Diquat @ 259 nm Paraquat Concentration 10X Diquat With No Significant Error

Plot Analysis at 316 nm

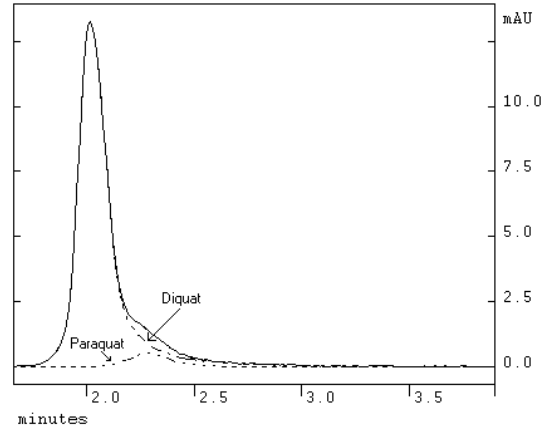| Library Index	t_R (min)	Height mAU	Width (sec)	Resolution
#2	2.015	13.26	8.8	—
#1	2.286	0.53	12.3	0.90

Figure 4. Plot Analysis of Paraquat and Diquat @ 316 nm Paraquat Concentration 10X Diquat

A significant error, such as an unexpected impurity, would result in a peak exceeding the noise threshold in the error analysis plot (Figure 5). The plot error analysis is not limited to the single wavelength of the reconstructed chromatogram plot. The error analysis is done as a comparison of the spectrum at each point in the chromatogram to the reconstructed spectrum from the MCA spectral analysis. This allows all wavelengths of interest to be evaluated and all impurities to be detected.

MCA's spectrum analysis (Figure 6) contains quantitative information and additional error analysis. The Similarity and Dissimilarity coefficients would differ significantly from 1.0 and 0.0 respectively, if there was a large error in the results. The results will automatically indicate a bad match if it applies (Figure 7).

In addition, the spectrum difference portion of the plot compares the current area spectrum (AU•s) to the reconstructed spectral sum based on the library spectra and presents this magnified difference (mAU•s). The difference spectrum is magnified and autoscaled to make the small differences between the target and reconstructed spectrum as visible as possible.
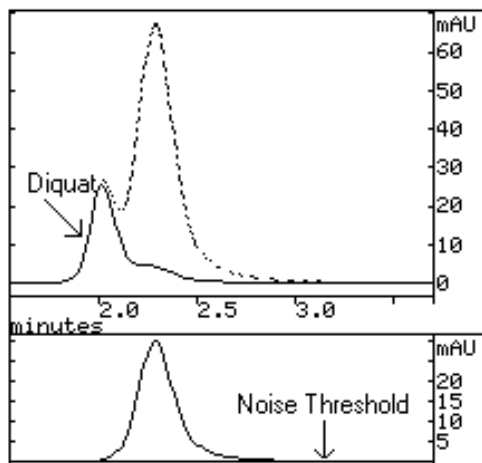

Figure 5. Plot Analysis (top) and Error Analysis (bottom) at 259 nm With an Unexpected Impurity Peak>Noise Threshold

During the MCA spectral decomposition, the wavelength range 335-367 nm is excluded since it contains no significant absorbance. This increases the accuracy of the results.

Spectrum Analysis over 215-367 nm, except 335-367

Library Index	Name	Amount
#1	Paraquat	9.95 µg
#2	Diquat	0.945 µg

Similarity: 1.00000
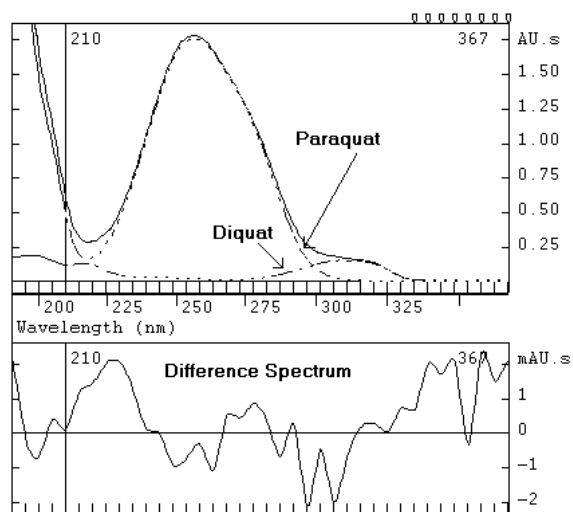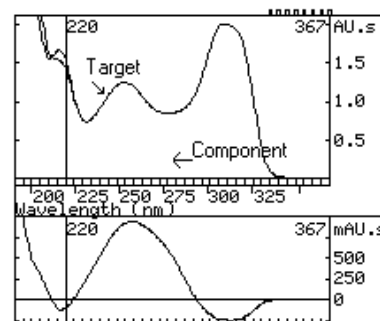Dissimilarity: 0.00150

Figure 6. Spectrum Analysis of Paraquat and Diquat No Significant Spectral Difference

Spectrum Analysis over 220-367 nm, except 335-367

Library Index	Name	Amount
#2	Diquat	12.32 µg

Similarity: 0.90226   Bad Match
Dissimilarity: 0.43119   0.00000024 (expected value)

Target Spectrum = Area Spectrum over Integration Region
Component Spectrum = MCA Decomposition limited to Diquat only

Figure 7. Spectrum Analysis over 220-367 nm, except 335-367 nm

The quantitative information is the same at both plot wavelengths (259 nm and 316 nm). These MCA results are compared to quantitative results from single wavelength multipoint calibrations, as shown in Table 2.

For the Paraquat and Diquat example, acceptable standard quantitation will be the result when one component is present at a high level and the other at a low level and quantitation is done at the optimum wavelength of the most concentrated analyte. Unacceptable quantitation will be the result when the low level compound is quantitated in the presence of the high level compound even at its optimum wavelength. MCA will provide accurate quantitative results in both cases.

Table 2 MCA Quantitation vs. Single Wavelength Multipoint Quantitation

Compound	Case	MCA*	Multipoint**
Paraquat	A	101.1%	103.4%
		±1.02%	±1.21%
	U	101.7%	158.0%
		±1.50%	±1.73%
Diquat	A	98.9%	98.5%
		±0.20%	±0.57%
	U	96.7%	142.0%
		±2.67%	±2.93%

*MCA library based on 10 μg injections of each compound
**Multipoint Range = 10 (1-10 μg injected),
R (paraquat) = 0.9984, R (diquat) = 0.9996
A = Acceptable Multipoint conditions
U = Unacceptable Multipoint conditions
Amounts Injected:
Paraqaut = 10.00 μg, Diquat = 1.00 μg, n=3
Paraqaut = 1.00 μg, Diquat = 10.00 μg, n=3

With these herbicides the presence of both in one analysis could be easily missed if diode array data were not properly evaluated. Typical diode array tests for purity could miss the small peak fused to the main peak, as they are often applied only to the peak heart region. MCA can also be used for assessing peak homogeneity by comparing an average spectrum to the spectrum at each point in the time range. This generates a Purity Report and the results would be the same as a plot analysis with the error analysis segment indicating where the impurities are found. Both types of results are produced as automations reports.

Conclusions

Multicomponent Analysis is a very powerful technique which provides accurate quantitative results from partially resolved peaks. The MCA process is fast and more accurate than conventional single channel integration techniques. In addition the purity assessment feature of MCA provides greater confirmation of peak identity and homogeneity than many other diode array purity evaluation techniques.

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