Multiple Wavelength Data Acquisition with the Vanquish Variable Wavelength Detector

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Key Words
Vanquish, VWD, UV-Vis, Absorption, Wavelength Switching

Goal
To explain the background of wavelength switching and demonstrate the use of the Thermo Scientific™ Vanquish™ Variable Wavelength Detector F for the separation of dyes.

Introduction
In UV-Vis absorption measurement, the selection of the best detection wavelength has a substantial impact on the analytical result. What are the criteria for a suitable wavelength selection? The main requirement for the wavelength choice is a sufficient absorption by the various analytes in the sample, combined with acceptable light transmittance by the mobile phase.[1] At detection wavelengths $\lambda \leq 220$ nm, most compounds absorb (near-universal detection), which is one reason why this detection wavelength area is frequently used.[2] Unfortunately, not only the analytes but also the mobile phases absorb in this range. Although pure solvents are mostly transparent in the typical wavelength range of a UV lamp, low concentrations of impurities with high ε (molar absorptivity) or higher concentrations of impurities with low ε values will create absorbance problems. Consistent removal of such impurities from solvents is therefore critical to reproducible chromatographic and analytical performance.[3] As a consequence, at low detection wavelengths the analysis becomes less rugged, system peaks have a greater chance to show up, and the baseline of a gradient separation is more prone to drift.[4] In addition, the absorption of the mobile phase will result in a background signal that directly contributes to baseline noise, and therefore adversely affects the limit of detection (LOD).[5]

When high specificity and precision is the goal of an assay, it makes sense to seek for alternative detection wavelengths $\lambda > 220$ nm. A requirement for that is the availability of a local absorption maximum (or minimum). There, not only is the absorption high but also the precision of the measurement. The main reason for that is the slope of the spectrum around the selected wavelength λ. At a local absorption maximum λ_{max}, the slope $m = 0$. Therefore, small deviations of the real λ from the nominal λ have only a minor impact on the respective peak area. When measuring in a spectrum flank, m is obviously large (red indication in Figure 1). Small λ differences therefore lead to a substantially reduced peak area precision.

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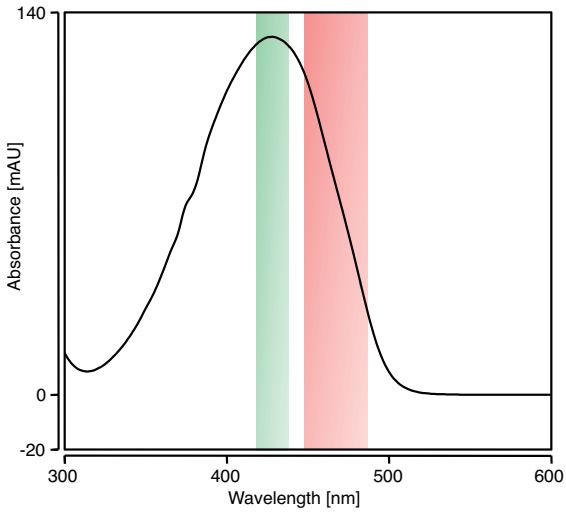

Figure 1. Absorption spectrum with indication on preferred (green) and improper (red) measurement wavelength selection.

Besides the pure wavelength accuracy of the detector, the previously mentioned impurities or bathochromic shift (caused by varying pH or organic content) can influence the absorbance spectrum. Although, depending on the characteristics of the influence, it is again generally desirable to measure at an absorbance maximum. This will, in most cases, reduce the extent of the peak area change and therefore result in better integration precision. In summary, it may make sense to change the detection wavelength during a chromatographic separation if high peak integration precision and ruggedness is a key requirement.

Variable wavelength detectors (VWDs) are UV-Vis absorption detectors commonly used in routine analysis. These detectors can typically acquire one wavelength at a time. For multiple wavelength acquisition, the monochromator in the detector optics must mechanically switch between the respective wavelengths. The Thermo Scientific Vanquish Variable Wavelength Detector F can simultaneously acquire up to four wavelength channels by continously switching the monochromator between the wavelength. Furthermore, it can do wavelength switching within one data channel. This technical note explains the background of the two approaches and discusses pros and cons on the basis of a separation of dyes.

Experimental

System

- Thermo Scientific™ Vanquish™ Flex System consisting of the following modules:
  - System Base Vanquish Flex (P/N VF-S01-A)
  - Quaternary Pump F (P/N VF-P20-A)
  - Split Sampler FT (P/N VF-A10-A)
  - Column Compartment H (P/N VH-C10-A)
  - Variable Wavelength Detector F (P/N VF-D40-A)
  - Semi-micro flow cell, 2.5 µL (P/N 6077.0360)
- Thermo Scientific™ Chromeleon™ Chromatography Data System, version 7.2, SR3 MUa
- All flow connections were established with Thermo Scientific™ Dionex™ Viper™ fingertight fittings.

Chromatographic Conditions

Standard	Approximately 10 µg/mL for all compounds, dissolved in gradient start solvent
Column	Thermo Scientific™ Hypersil GOLD aQ™, 2.1 × 100 mm, 1.9 µm
Mobile Phase	A: 20 mM ammonium acetate, pH 7.4 adjusted with 8 N sodium hydroxide B: Acetonitrile
Flow Rate	0.5 mL/min

Gradient

Time	%B
0.0	3
8.0	50
8.1	100
8.5	100
8.6	3
25.0	3

Column Temperature	30 °C, still air
Injection Volume	3 µL
Run Time	25 min

Detector Settings

Single channel acquisition, common settings

Data Collection Rate	20 Hz
Response Time	0.2 s

UV and VIS lamp were operated simultaneously.

Single channel with single wavelength

Wavelength for single wavelength acquisition 254 nm

Single channel with multiple wavelengths

Wavelength switching timetable

Time	Wavelength [nm]
0.00	427
2.10	521
2.80	610
3.50	508
3.96	484
4.90	625
6.22	530
6.78	545
7.62	554

Multichannel acquisition

Channel 1	254 nm
Channel 2	504 nm
Channel 3	550 nm
Channel 4	610 nm
Data Collection Rate	1 Hz
Response Time	5 s

Variable Wavelength Detectors

VWDs are typically dual-beam variable wavelength photometers with one measurement and one internal reference beam. Depending on model and manufacturer, these detectors can measure at one or a few detection wavelengths simultaneously. Figure 2 shows the optical layout of the Vanquish VWD.

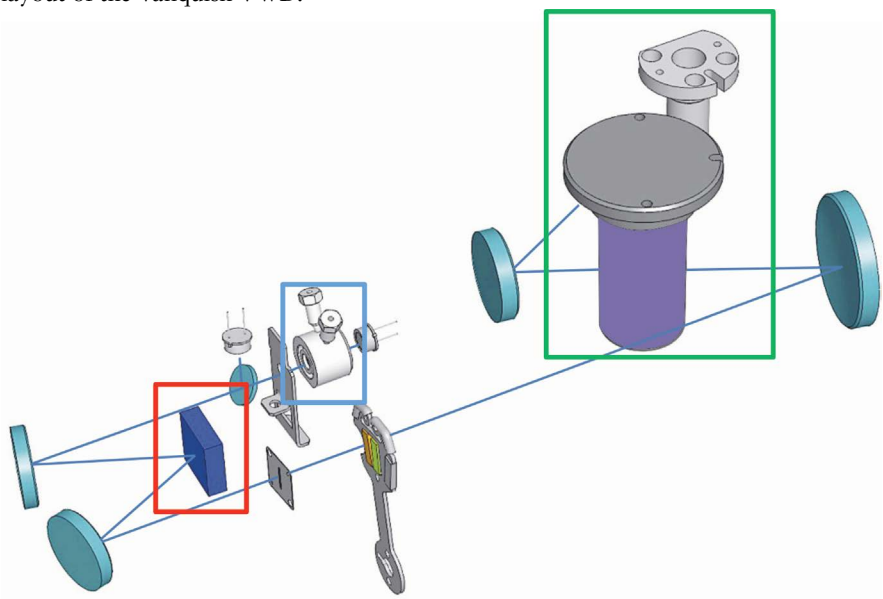

Figure 2. Optical bench design of the Vanquish VWD. The grating (red) is located between lamps (green) and flow cell (blue). Therefore, only a fraction of the dispersed light passes through the flow cell.

Following the light path from the lamp to the flow cell immediately shows that the light passes the monochromator before it enters the flow cell. Due to the light dispersion of the monochomator, it is obvious that only a portion of the light passes through the flow cell and therefore interacts with the sample. A different detection wavelength is selected by repositioning the monochromator. The simultaneous acquisition of more than one wavelength therefore requires the repeated positioning of the optical grating in the following fashion:

Collect data at λ_1 → move grating → collect data at λ_2 → move grating → collect data at λ_3 → move grating → collect data at λ_1 → …

After these multiple steps of data collection and grating movement, the detector has only collected 2 data points for λ_1 and one each for λ_2 and λ_3.

In addition to the grating, the Vanquish VWD has two optical filters that take part in the wavelength switching process. Depending on the measuring wavelength, these filters automatically move into the light path, thus ensuring optimal measuring results over the entire wavelength range. With a specific selection of wavelengths, the filter may therefore change with each grating repositioning during multiple wavelength acquisition.

As moving the grating and filters takes some time and additional time is required to stabilize them before the next data point acquisition, some limitations of multiwavelength acquisition with VWDs become obvious:

- The data collection rate for Vanquish VWD F detectors is lower than 5 Hz when simultaneously acquiring more than one wavelength channel. In single-channel mode, the Vanquish VWD can acquire data up to 250 Hz.
- Baseline noise is higher during multichannel operation. This is mainly caused by remaining vibration during data acquisition and only short effective measurement time.
- Although the involved optical components are designed for high ruggedness, permanent multichannel acquisition with VWDs causes them to wear. Thermo Fisher Scientific therefore recommends the use of VWD multichannel acquisition for only a limited time, e.g. during method development.

The Vanquish VWD offers an alternative to multichannel acquisition. The wavelength can easily be changed within one channel. This divides the chromatogram in segments with λ_1, for instance, during the elution of peak 1 and λ_2, for instance, during the elution of peak 2. As the detector cannot acquire data during the grating movement event and the attenuation coefficient (old: extinction coefficient) changes with the selected wavelength, it should only be done while the flow cell contains no relevant peak. Compared to HPLC separations, UHPLC conditions significantly shorten the available time window for the required grating movement,[6] so the time needed for this should be as short as possible.

Results and Discussion

Single Wavelength Acquisition

Figure 3 shows a separation of a dye standard mixture, detected at 254 nm. Table 1 identifies the peaks and their related retention time and area RSDs from six consecutive runs. The retention time precision is very good, indicating stable chromatographic conditions. The peak area however is 10–20 times less precise. The reason for this is the use of a single detection wavelength for a separation of compounds with very different absorption spectra.

As an example, Figure 4 shows the absorption spectra of New Coccine and Fast Green. The ordinate represents the absorption relative to 254 nm, the detection wavelength for the chromatogram in Figure 3. The red line indicates this wavelength; the green line indicates the proposal for a wavelength change. For New Coccine, the main reasons to change are better specificity and improving the peak area precision. Although not at an absorption maximum for best detection precision, relatively high absorption is present at 254 nm. At 508 nm however, only a limited number of analytes has relevant absorption. In addition, the peak area precision will benefit from measuring at an absorption maximum. For Fast Green, the absorption at 254 nm is more than 14 times lower than at 625 nm. When the analysis is done at this wavelength, the results will most likely benefit from both higher absorption and better precision.

Table 1. Retention time precision of dye standard separation (single-channel detection at 254 nm, six consecutive runs).

No.	Compound	Retention Time RSD [%]	Area RSD [%]
1	Tartrazine	0.19	0.72
2	Amaranth	0.04	0.75
3	Indigo Carmine	0.06	0.55
4	New Coccine	0.06	0.67
5	Sunset Yellow	0.05	0.73
6	Fast Green	0.04	0.67
7	Erioglaucine Na_2	0.03	0.60
8	Erythrosine B	0.03	0.65
9	Cyanosine	0.04	0.74
10	Bengal Rose	0.03	0.74

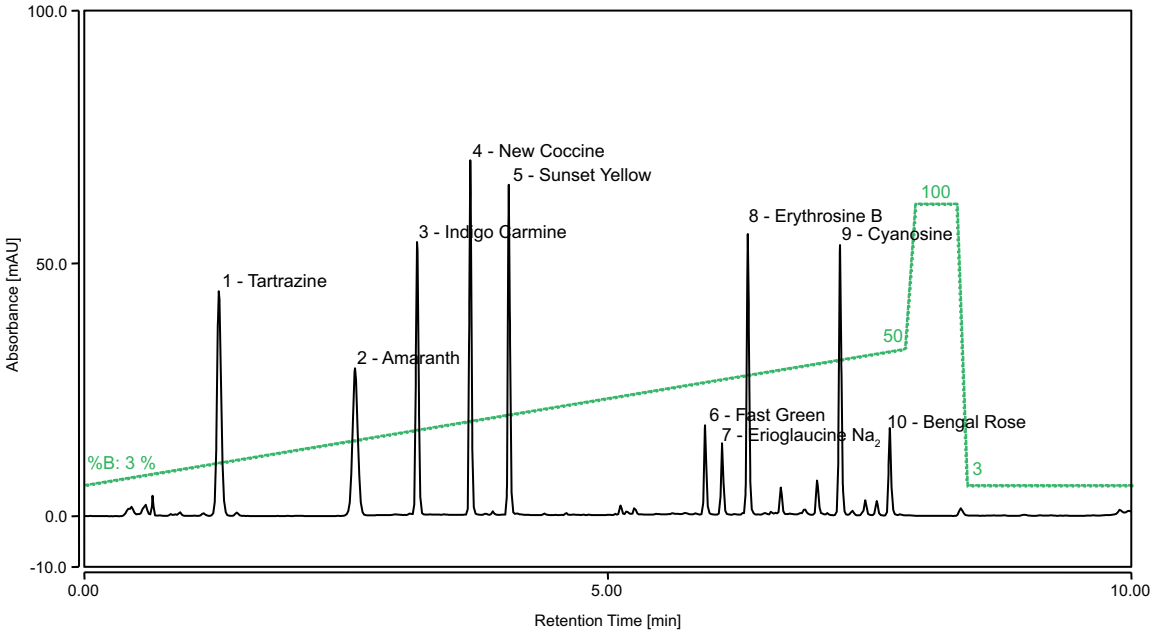

Figure 3. Separation of a dye standard at 254 nm. Nonlabeled peaks are impurities.

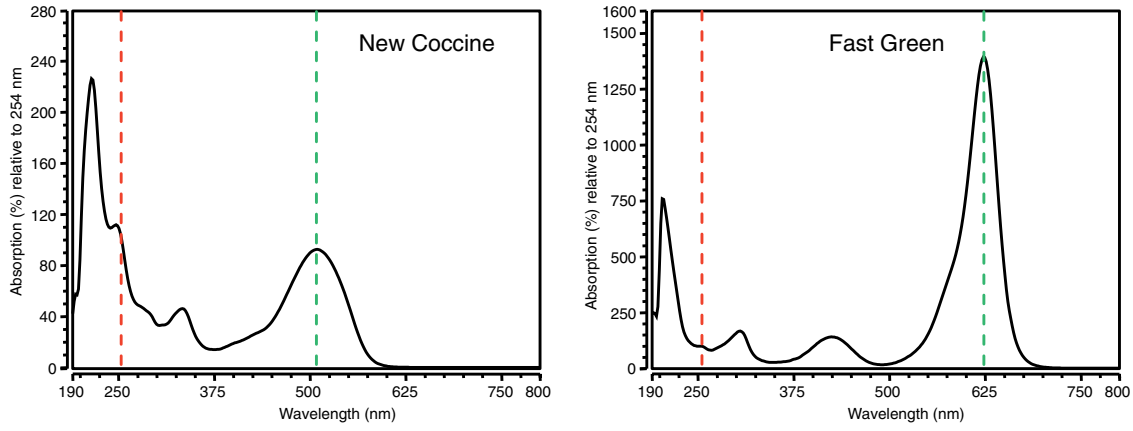

Figure 4. Absorption spectra of New Coccine and Fast Green, relative to the absorption at 254 nm (equivalent to 100%).

Simultaneous Acquisition of Four Channels

The Vanquish VWD F detector offers the option to simultaneously acquire data on up to four signal channels. The technology is described in the *Background* section. For the dye application, four signal channels mean a compromise, as the spectra of the 10 analytes are very different. After reviewing all spectra, we chose 254 nm, 504 nm, 550 nm, and 610 nm. Figure 5 shows an overlay of the 254 nm channel as part of a four-channel acquisition and single wavelength acquisition as already shown in Figure 3.

The overlay shows that with multichannel acquisition, peak heights are reduced and delayed. The reason for this is that the simultaneous acquisition of the four chosen wavelength channels decreases the data collection rate to 1 Hz. The chromatogram, however, requires a higher data rate to accurately depict and integrate the peaks. For best integration of this chromatogram, we selected 20 Hz and a related response time of 0.2 s. Response time is a parameter to improve the signal-to-noise (S/N) ratio. In addition, the response time also has an impact on the peak height and width, the chromatographic resolution, and the retention time, if not properly set. For the multichannel acquisition, the data rate is too low, which also results in a too long response time setting of 5 s for this separation. Lower, broader, and delayed peaks are a consequence.

Table 2 lists S/N differences of single and multichannel acquisition. Except for the first peak, the single wavelength acquisition is clearly superior when only comparing acquisitions at 254 nm. When the best suitable wavelength is selected, the multichannel acquisition achieves for most peaks a better S/N.

Table 2. S/N performance comparison of single and multichannel acquisition.

No.	Compound	S/N at 254 nm, single wavelength	S/N at 254 nm, multichannel	S/N, multichannel best wavelength
1	Tartrazine	2070	3120	2629 (254 nm)
2	Amaranth	1890	502	2201 (550 nm)
3	Indigo Carmine	4286	1100	851 (550 nm)
4	New Coccine	5129	5984	2332 (504 nm)
5	Sunset Yellow	4366	5339	4880 (504 nm)
6	Fast Green	1185	555	3855 (610 nm)
7	Erioglaucine Na_2	863	226	4265 (610 nm)
8	Erythrosine B	3235	1074	4416 (504 nm)
9	Cyanosine	2477	1270	6773 (550 nm)
10	Bengal Rose	1043	531	1189 (550 nm)

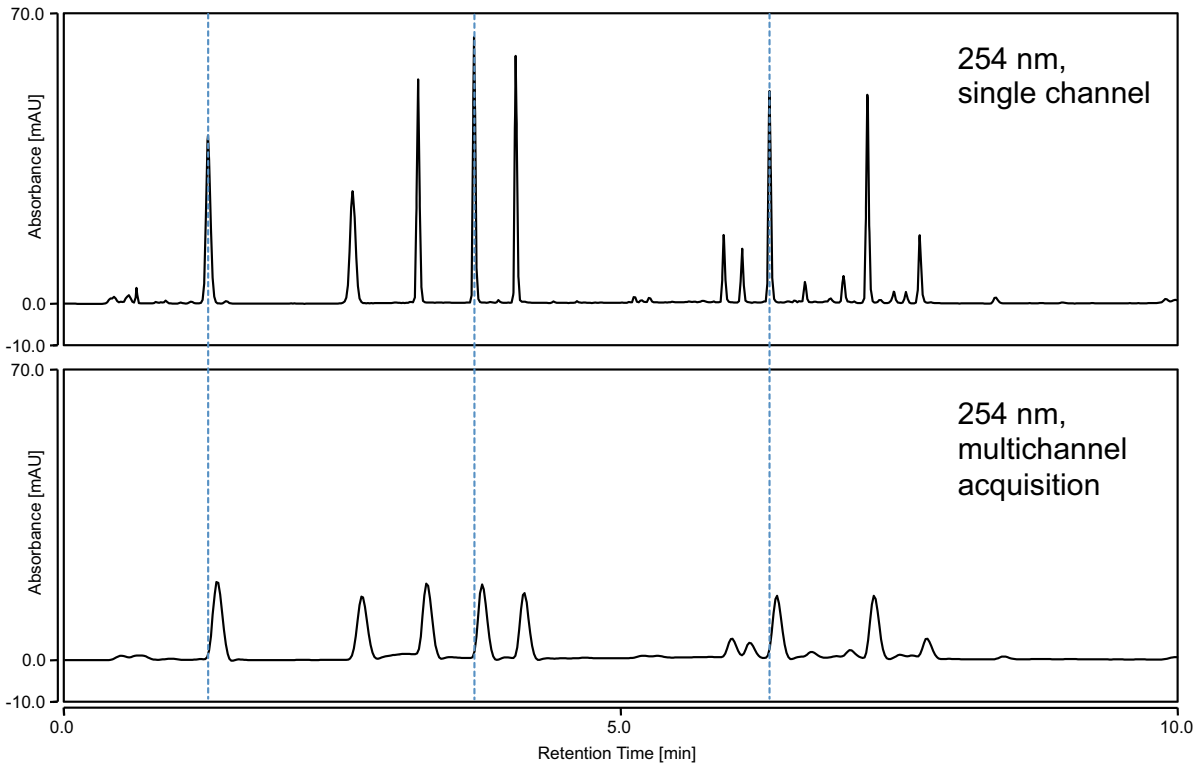

Figure 5. Overlay of acquisitions at 254 nm: single data channel (top) and 254 channel as part of the simultaneous acquisition of four (254, 504, 550, and 610 nm) channels (bottom).

Single-Channel Acquisition with Multiple Detection Wavelengths

With the Vanquish Variable Wavelength Detector, the detection wavelength can be changed at any time of the chromatographic run. This approach has the potential to combine the advantages of single and multichannel data acquisitions, e.g.:

- High data collection rates
- Low baseline noise, monochromator movements only as timed events
- Improved peak area precision
- Better signal-to-noise ratios

With a VWD, a change of the detection wavelength has an impact on the baseline. During grating movement, no data acquisition takes place. After the new target wavelength is reached, the Vanquish VWD offers three options for how to continue data acquisition. For all settings, the time required for wavelength switching is extremely short for best compatibility with UHPLC separations.

Figure 6 compares the baseline settings Free, Autozero, and Baseline Append (from left to right). Similar to the beginning of the chromatogram, Autozero sets the signal to zero. Free does not set to zero. Instead, the baseline jumps up or down, e.g. as a consequence of mobile phase absorption. This mode can be useful to monitor the mobile phase quality.[7] Baseline Append ensures that the baseline level does not change after a wavelength change during the run and is the default mode.

Figure 7 shows a chromatogram measured on a single channel with multiple wavelength switches and Baseline Append compared to the chromatogram in Figure 3 without wavelength switching. The retention times of all analyte peaks perfectly match. Particularly, peaks 6 to 10 show improved responses. Table 3 summarizes the S/N performance obtained with both approaches. For peaks 1 and 5, the S/N clearly decreases with wavelength switching. The selected wavelengths were partially chosen to achieve a better precision and specificity of the measurement (see Figure 4). Both UV and Vis lamps operated simultaneously to achieve the highest light intensity between 345 nm and 670 nm, but light is very intense at 254 nm. This can be beneficial for the noise performance when measuring at this wavelength compared to longer wavelengths. For other peaks, the aspect of higher absorption of the analyte is dominating, leading to better S/N. The biggest difference is observed for Erioglaucine Disodium Salt (peak 7, Erioglaucine Na_2) with more than 14 times better S/N.

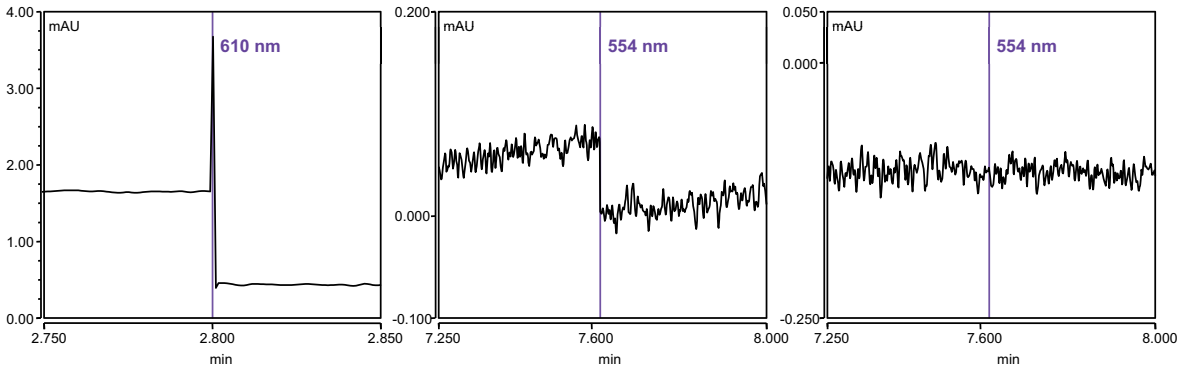

Figure 6. Comparison of baseline behavior settings, from left to right: Free, Autozero, Baseline Append.

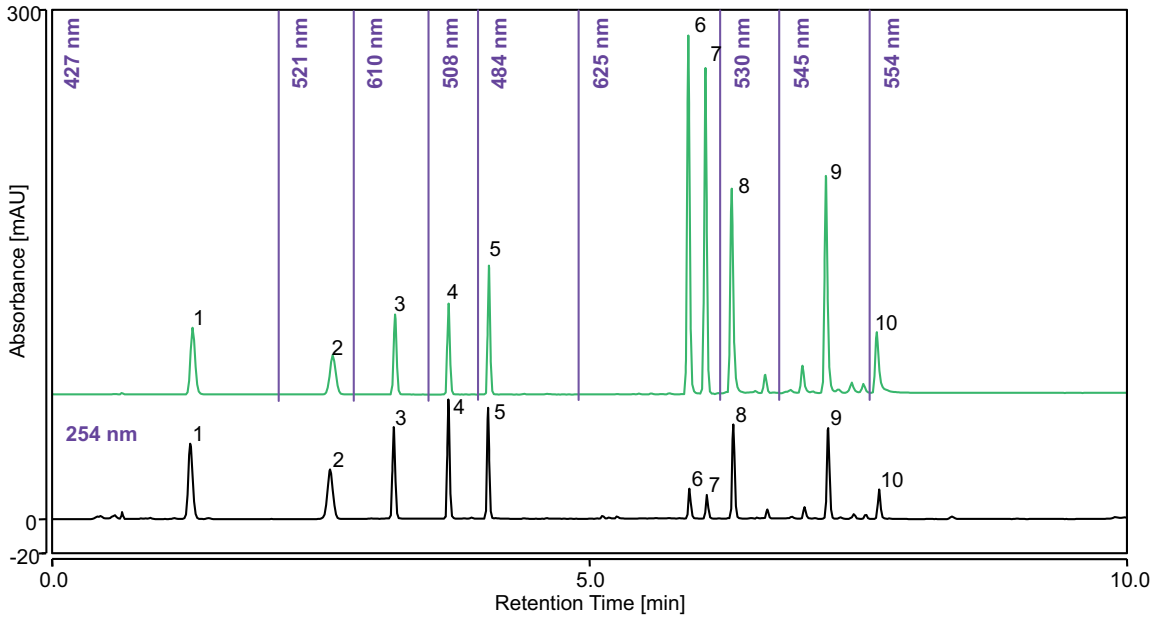

Figure 7. Overlay of single data channel acquisitions, measured at 254 nm (black chromatogram) and with wavelength switching (green chromatogram).

Table 3 also compares the area precision of the two approaches. For all analytes, the precision of the measurement benefits from the customized wavelength setting. For Indigo Carmine, the area precision improves by a factor of nine.

Table 3. S/N performance comparison and area precision comparison of single channel data acquisition, at 254 nm, and with wavelength switching.

No.	Compound	S/N		Area RSD, six consecutive replicates [%]	
		Single channel at 254 nm	Single channel, wavelength switching	Single channel at 254 nm	Single channel, wavelength switching
1	Tartrazine	2070	812	0.72	0.25
2	Amaranth	1890	1279	0.75	0.21
3	Indigo Carmine	4286	4192	0.55	0.06
4	New Coccine	5129	4630	0.67	0.12
5	Sunset Yellow	4366	3136	0.73	0.13
6	Fast Green	1185	13710	0.67	0.09
7	Erioglaucine Na_2	863	12726	0.60	0.09
8	Erythrosine B	3235	6811	0.65	0.37
9	Cyanosine	2477	6982	0.74	0.42
10	Bengal Rose	1043	1953	0.74	0.25

Improved Specificity for Real Samples

Figure 8 and Figure 9 compare results from real samples, overlaying single-channel acquisitions at 254 nm with wavelength switching. For both overlays, wavelength switching provides a much better specificity by screening out unwanted impurity or matrix peaks (green chromatograms, top). As demonstrated for the injections of standards, the Erioglaucine Na_2 peak is much higher, improving the sensitivity of the test.

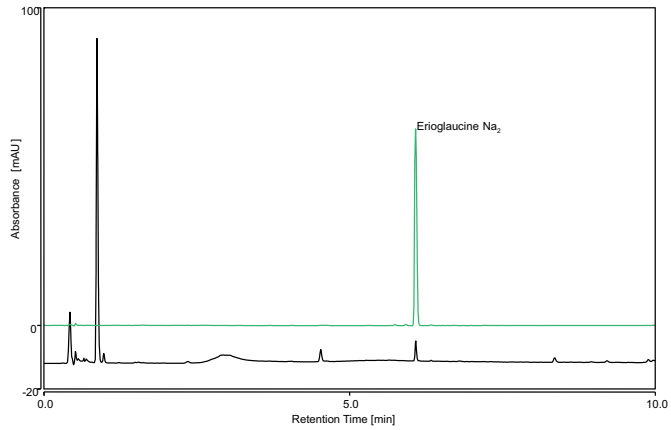

Figure 8. Overlay of two injections of an electrolyte sports drink sample, displayed with 10% signal offset. Obtained by single data channel acquisition, without (black chromatogram, bottom, at 254 nm) and with (green chromatogram, top) wavelength switching.

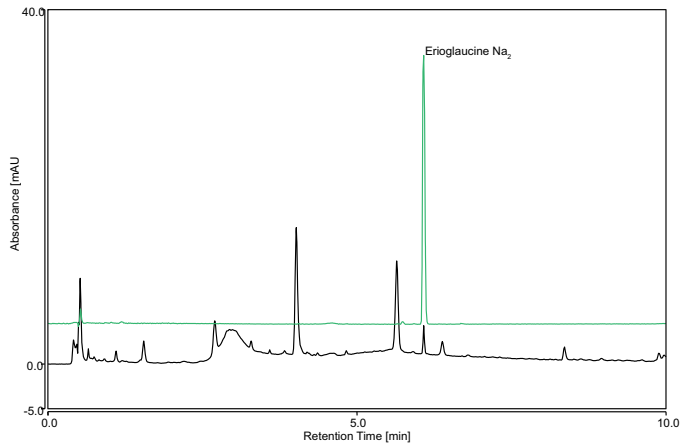

Figure 9. Overlay of two injections of a diluted woodruff sirup sample, displayed with -10% signal offset. Obtained by single data channel acquisition, without (black chromatogram, bottom, at 254 nm) and with (green chromatogram, top) wavelength switching.

Conclusion

The Vanquish VWD supports the acquisition of multiple wavelengths during a chromatographic run. The recommended approach is to acquire a single data channel and to switch the wavelength in between the peaks according to the requirements of the analyte. In contrast to acquiring data at only one wavelength, or at several wavelengths in multichannel mode, this approach combines the advantages, like high data collection rates, better signal-to-noise ratios, and improved peak area precision. For a separation of dyes, the switching of wavelengths improves the peak area precision up to a factor of nine and the S/N up to a factor of 14. The analysis of real-life samples demonstrates that wavelength switching also improves the specificity of the analysis.

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