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Demonstrating Superior Linearity: The ACQUITY UPLC Photodiode Array Detector (PDA)

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Abstract

This application note demonstrates a wide linear dynamic range with 5.0% deviations typically above 2.5 AU using ACQUITY UPLC System and ACQUITY UPLC Photodiode Array Detector. Linearity tests of a main component and its 0.01% impurity were shown to be easily validated.

Introduction

There are two characteristics that determine the value of a detector: performance at low noise levels and a wide linear dynamic range. These are crucial for applications such as quantitative impurity analysis to analyze both a large amount of parent compound and low-level impurity peaks (<0.05%) within the same chromatogram, and for food safety and environmental applications where the levels of the compounds of interest can vary dramatically. Designed for the resolution, speed, and sensitivity of Ultra Performance LC (UPLC), the ACQUITY UPLC Photodiode Array (PDA) Detector is ideal for such tasks, with a linear dynamic range that extends over more than four orders of magnitude and is in excess of 2.0 AU. Combined with low noise characteristics,[1] the ACQUITY UPLC PDA allows for the validation of linearity for both high-and low-level components (<0.01%) and quantitation over a wide concentration range.

Experimental

Method for Detector Linearity

System:	ACQUITY UPLC System with the ACQUITY UPLC PDA Detector and Empower Software
Flow cells:	125 mg/mL: Analytical Cell 50 mg/mL: High Sensitivity Cell
Mobile phase:	A: Methanol

	B: Methanol with Propyl Paraben
Gradient:	1% to 97% B in 4% steps, with a 3 min duration
Flow rate:	1 mL/min
Pressure:	4,000 psi (restrictor)
Wavelength:	254 nm
Bandwidth:	1.2 nm
Data rate:	10 Hz
Time constant:	0.2 sec

Prilocaine Impurity Method

System:	ACQUITY UPLC System with PDA (Analytical Flow Cell) and Empower
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7 µm
Injection volume:	2 µL, Full Loop
Sample:	Prilocaine: 2 mg/mL (100%) o-Toluidine: 0.2 µg/mL (100%)
Mobile phase:	A: 75/25 10 mM Ammonium B: Bicarbonate pH 9.5/Acetonitrile
Flow rate:	800 µL/min
Temp.:	40 °C

Weak wash:	1000 µL 75/25 Water/Acetonitrile
Strong wash:	200 µL 25/75 Water/Acetonitrile
Wavelength:	230 nm
Bandwidth:	1.2 nm
Data rate:	20 Hz
Time constant:	0.1 sec

(2,4-Dichlorophenoxy) Acetic Acid Method

System:	ACQUITY UPLC System with PDA (Analytical Flow Cell)and Empower
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7 µm
Injection volume:	2 µL, Full Loop
Sample:	2,4-D in 50/50 Water/Acetonitrile 0.410 to 800 µg/mL
Mobile phase:	A: 70/30 Water/Acetonitrile B: 0.1% Formic acid
Flow rate:	650 µL/min
Temp.:	35 °C
Weak wash:	800 µL 75/25 Water/Acetonitrile
Strong wash:	200 µL 20/80 Water/Acetonitrile

Wavelength:	230 nm
Bandwidth:	1.2 nm
Data rate:	10 Hz
Time constant:	Normal

Results and Discussion

ACQUITY UPLC PDA Linearity

The linearity of a detector is governed by the degree of stray light. The less stray light that is present in an optical detector, the wider its linear dynamic range. For maximum linearity performance, the ACQUITY UPLC PDA has two low-light level flow cell options for use with 2.1 mm i.d. columns: the analytical flow cell with a 10 mm path length, and the high-sensitivity flow cell with a 25 mm path length. Figures 1 and 2 demonstrate the linear ranges for the analytical and high sensitivity flow cells, respectively. Both flow cells typically have 5.0% deviation values above 2.5 AU, while deviations at 2.0 AU are typically below 2.0% (with a specification of less than 5.0%).

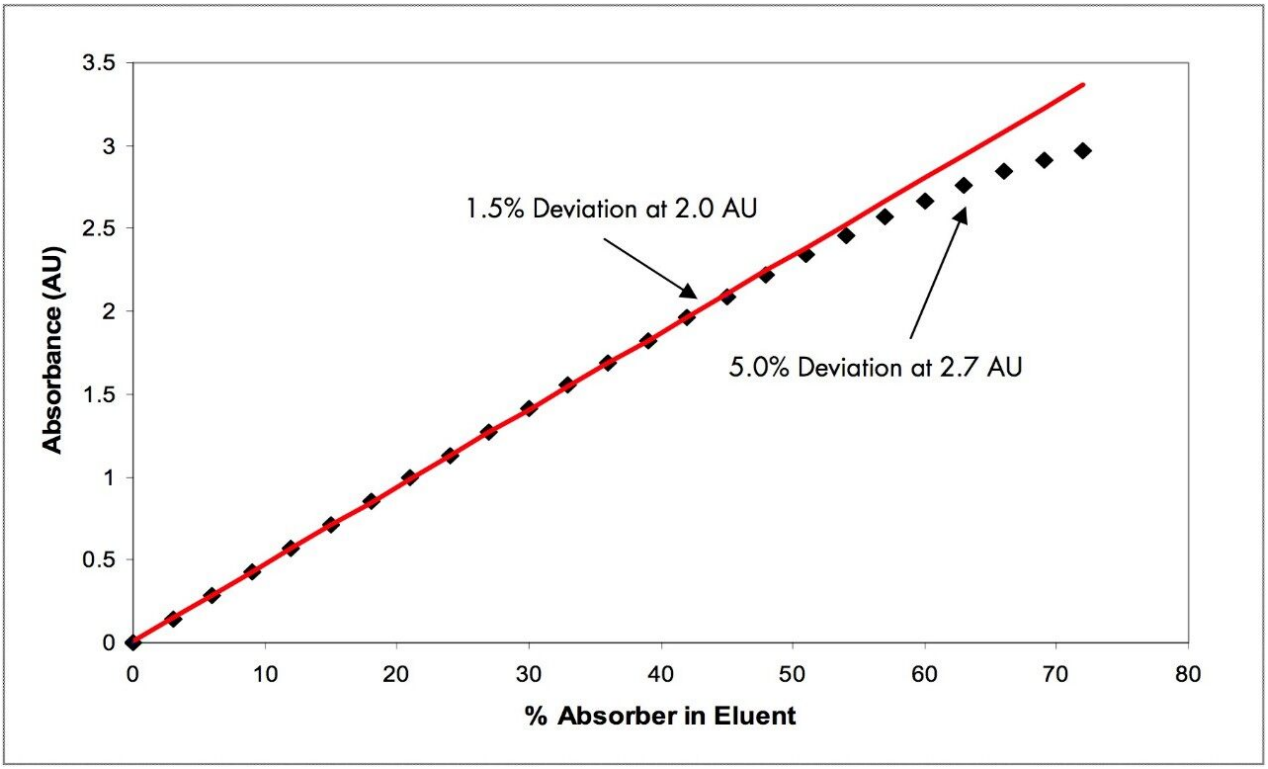

Figure 1. ACQUITY UPLC PDA linearity plot with % deviation values for the analytical flow cell.

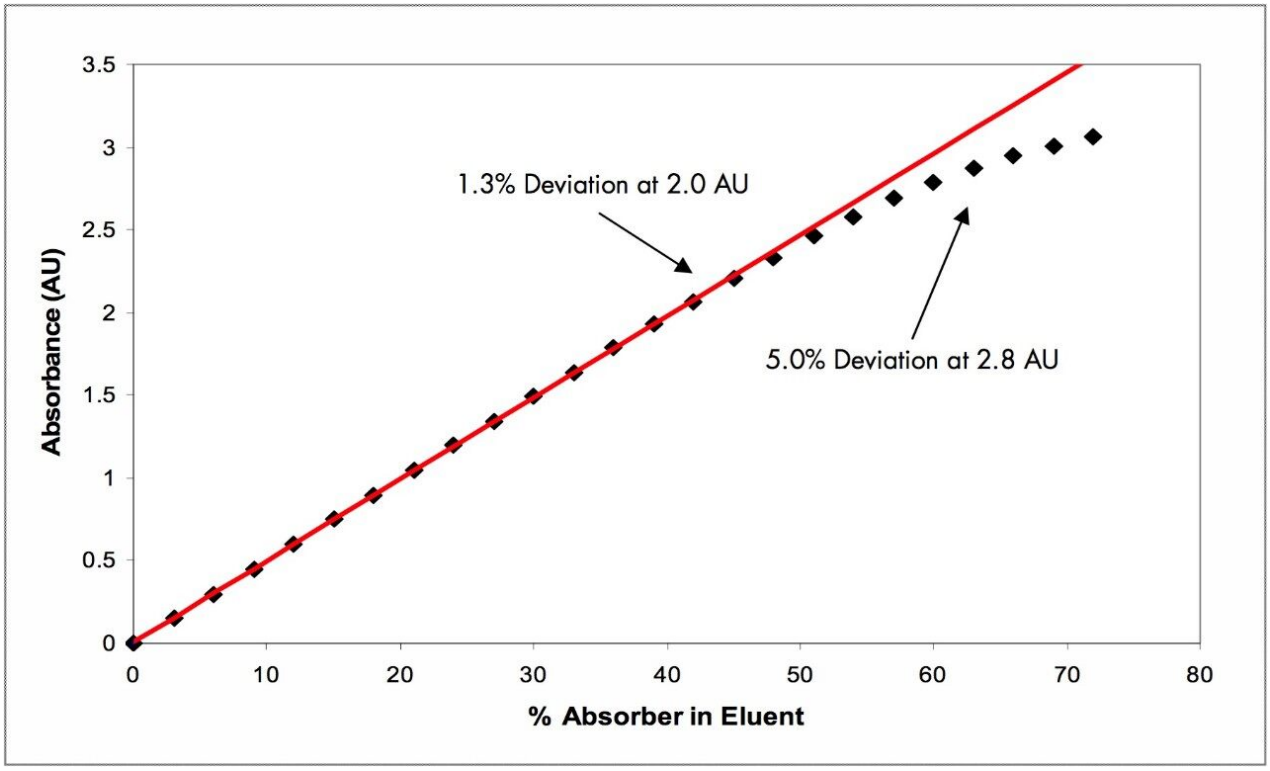

Figure 2. ACQUITY UPLD PDA linearity plot with % deviation values for the high sensitivity flow cell.

Linearity and Quantitative Impurity Analysis

It is important for quantitative impurity analysis to have a detector with a wide linear dynamic range to accurately quantitateboth the main component and impurities in the same chromatogram, and to meet validation requirements for linearity. The linear range of the main component must be validated[2] from 80%–120%, with typical acceptance criteria for R^2 values in excess of 0.9995 for the calibration curve. For quantitation of impurities, linearity must be validated over an extended range as impurity levels may be more susceptible to fluctuations. Typical acceptance criteria for the linearity of impurities are R^2 values in excess of 0.995 for the calibration curve. To demonstrate these validation requirements, Prilocaine and its impurity, o-Toluidine (Figure 3) were analyzed in duplicate at seven concentration levels. The validated range was 80%–100% for prilocaine, and 50%–150% for o-toluidine. The acceptance criteria were met as the prilocaine R^2 value was 0.9998 and the o-toluidine R^2 value was 0.998 (Figure 4).

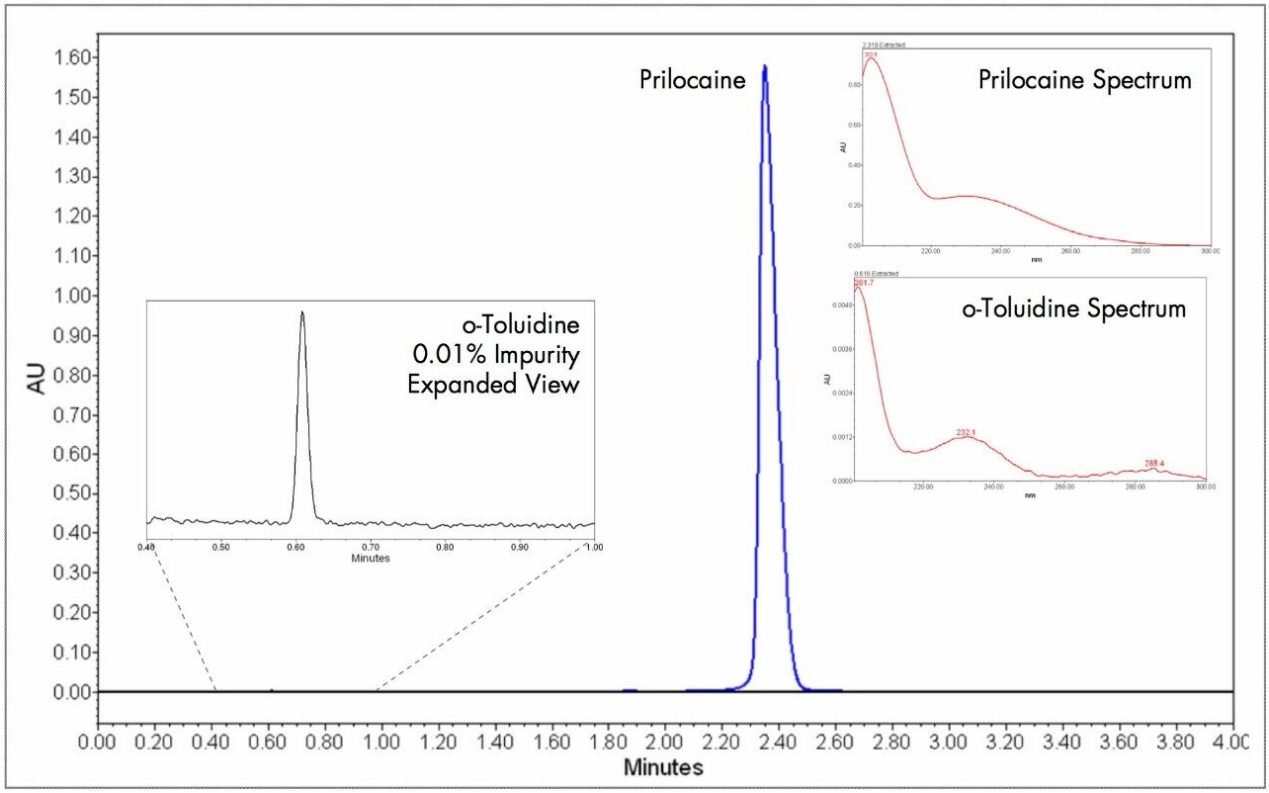

Figure 3. Prilocaine standard spiked with an impurity (o-toluidine) at 0.01%.

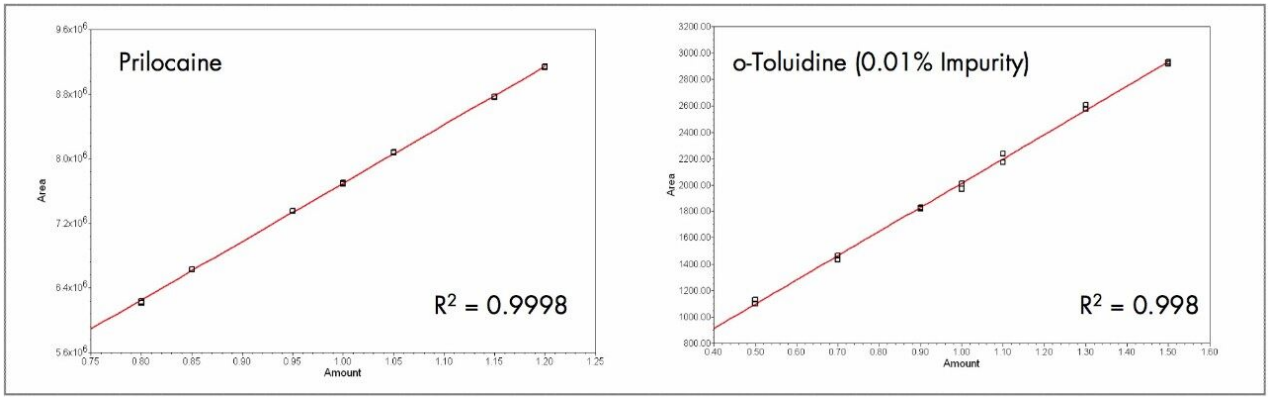

Figure 4. Linearity plots of prilocaine (main component) and o-toluidine (impurity) demonstrate good linearity.

Linearity and Variable Analyte Concentrations

For applications in food safety and environmental analysis, it is often necessary to quantitatecomponents across many orders of magnitude. With conventional detection approaches, several calibration plots over the

range are usually required. The ACQUITY UPLC PDA, however, is linear over more than four orders of magnitude, allowing quantitation from a single calibration curve. To demonstrate, a series of ten dilutions from 0.410 to 800 µg/mL of (2,4-dichlorophenoxy)acetic acid (2,4-D) standard were injected in six replicates to create a calibration curve (Figure 5). A 1/x weighting was chosen to reflect the variation of precision (standard deviation) over the wide concentration range.[3] For curves over a wide range such as this, R^2 is not a good indicator of linearity. Therefore, the % deviation from the curve (residuals) was plotted and examined for trends. The % deviation plot in Figure 6 does not exhibit trending at either end of the concentration range, indicating good linearity. Table 1 lists the average % RSDsat each level. The lowest level is near the limits of quantitation and thus has higher scatter of the residuals and a higher % RSD. Also shown is the average % deviation at each level as less than 2.0%.

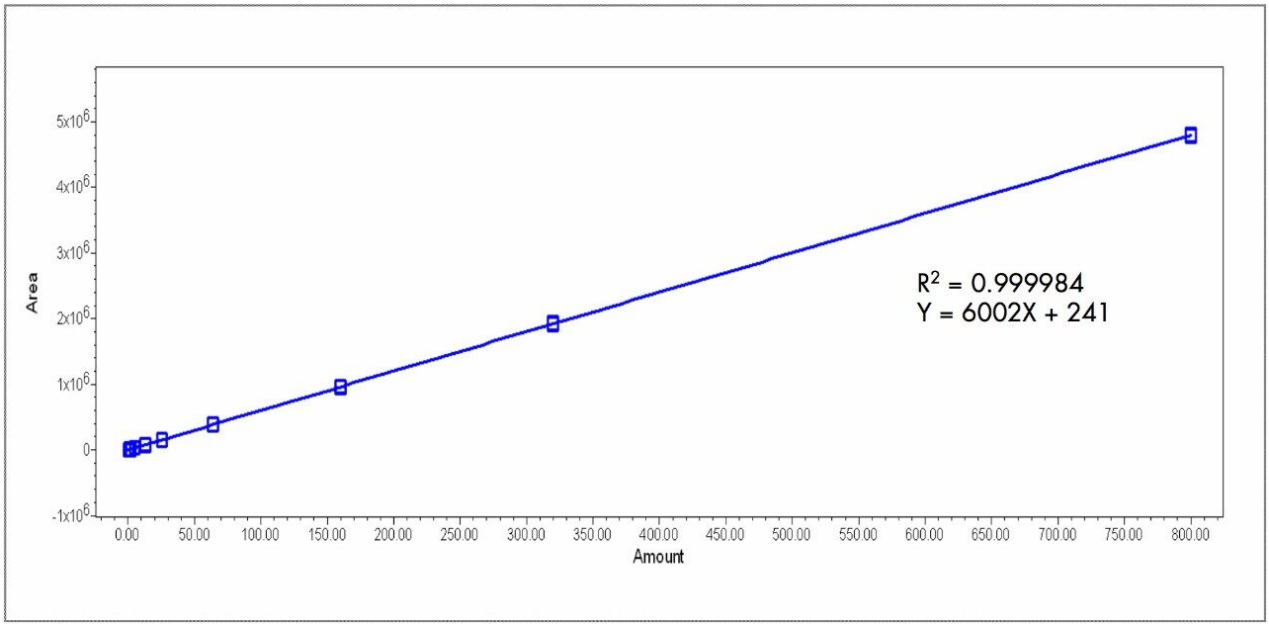

Figure 5. Calibration plot for (2,4-dichlorophenoxy)acetic acid from 0.410 to 800 µg/mL.

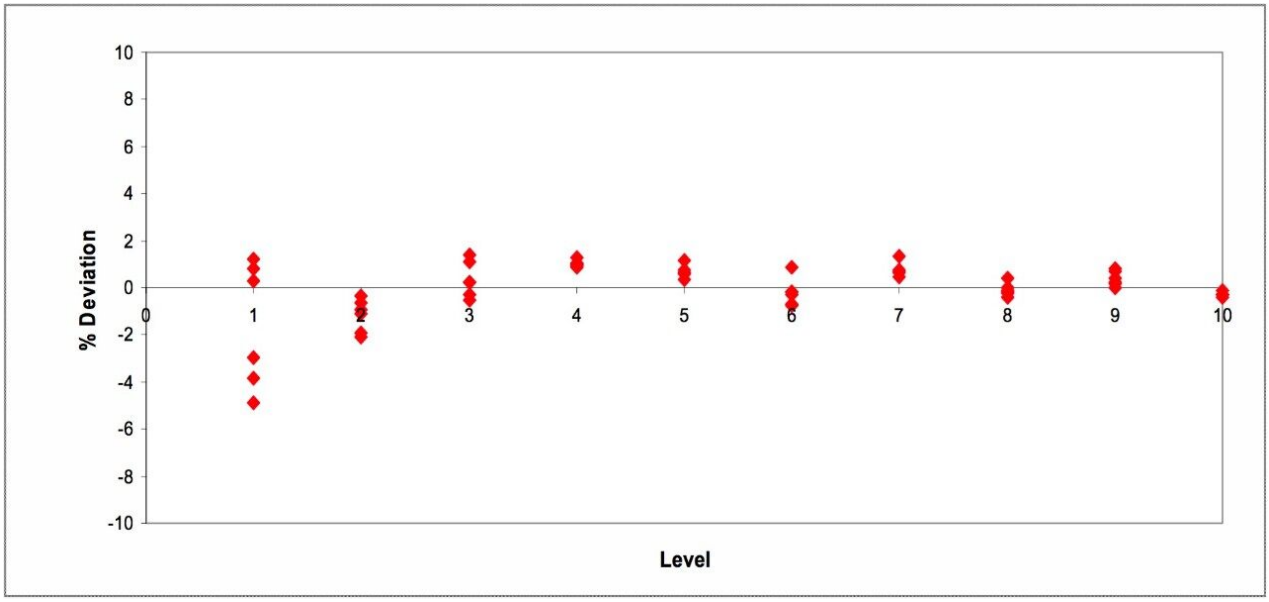

Figure 6. Plot of the % deviation from the calibration curve of 2,4-D from 0.410 to 800 µg/mL.

Level	Concentration (µg/mL)	% of High Standard	% RSD of Peak Area	Average % Deviation
1	0.410	0.0512	2.44	-1.56
2	1.02	0.128	0.67	-1.43
3	2.05	0.256	0.75	0.36
4	5.12	0.640	0.14	1.03
5	12.8	1.60	0.26	0.69
6	25.6	3.20	0.58	-0.21
7	64.0	8.00	0.30	0.77
8	160	20.0	0.28	-0.09
9	320	40.0	0.32	0.39
10	800	100	0.18	-0.21

Table 1. Average peak area % RSDs and average % deviation from the curve for the calibration of 2,4-D from 0.410 to 800 µg/mL.

Conclusion

Integral to the the superior performance of the ACQUITY UPLC System, the ACQUITY UPLC Photodiode Array Detector demonstrates a wide linear dynamic range with 5.0% deviations typically above 2.5 AU. Linearity tests of a main component and its 0.01% impurity were shown to be easily validated. A linear range in excess of four orders of magnitude makes the ACQUITY UPLC PDA ideal for all quantitative applications, from the routine to the most challenging.

References

1. Jenkins T. ACQUITY UPLC with PDA Detection: Determining the Sensitivity Limits of Oxybutynin and Related Compounds. 2006 Mar; Waters Application Note 720001595EN < https://www.waters.com/webassets/cms/library/docs/720001595en.pdf> .
2. General Chapter <1225>: Validation of Compendialmethods. United States Pharmacopeia(USP) XXIII, National Formulary, XVIII. 1995:1710–1612.
3. ErmerJ, Miller JH. Method Validation in Pharmaceutical Analysis: A Guide to Best Practice. 2005.

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