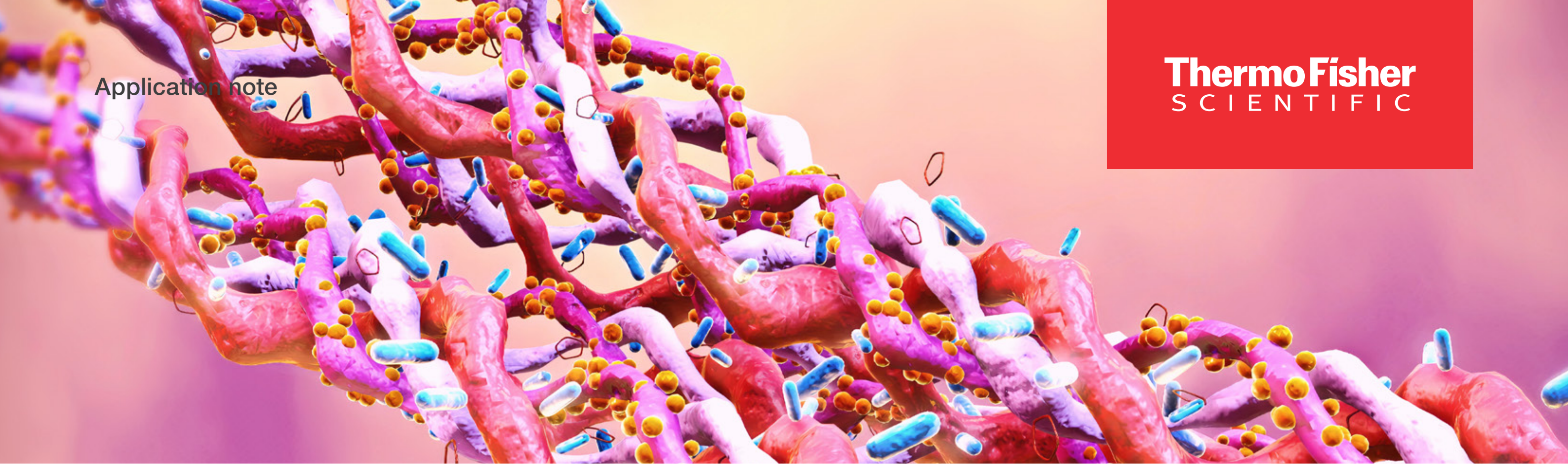

Application note

NanoDrop Ultra Acclaro Phenol and Protein Contaminant Identification

Introduction

The Thermo Scientific™ NanoDrop™ Ultra Microvolume UV-Vis Spectrophotometer and Fluorometer utilizes the Thermo Scientific™ Acclaro™ Sample Intelligence Technology, featuring Contaminant Identification (ID), which detects the presence of common contaminants in nucleic acid and protein samples. Traditionally, the nucleic acid purity ratios A260/A230 and A260/A280 serve as the primary resource for quantifying sample purity. The acceptable purity ratio ranges for “pure” nucleic acids are listed in Table 1. In some cases, purity ratios may be within the “pure” range when a contaminant is present. This limitation introduces concerns as the nucleic acid may seem pure, but contaminants will artificially inflate the sample concentration, leading to downstream reaction failures.

Nucleic acid	A260/A280	A260/A230
DNA	~1.8	1.8 – 2.2
RNA	~2.0	1.8 – 2.2

Table 1. Acceptable purity ratio ranges for “pure” nucleic acids using a blank and sample buffer at the same pH and ionic strength.

To circumvent the limitation of purity ratios, the built-in Acclaro Contaminant ID feature in the NanoDrop Ultra instrument analyzes individual sample spectra using a reference spectral library and chemometric algorithms. This technology allows the software to make predictions about the existence of contaminants and how much of that contaminant is present, and provide a corrected sample concentration (see Figure 1).

	ng/µL	A260/A280	A260/A230	A260	%CV
● Original	295.300	1.45	0.45		
● Corrected	240.7			-	2.35
● Protein	-			0.96	12.79

Figure 1. Acclaro Contaminant ID feature identified protein as a contaminant in a dsDNA preparation. The original, uncorrected spectrum is in green, the corrected spectrum is in purple, and the contaminant spectrum is in orange.

There are several contaminants that are identified using the Thermo Scientific™ Acclaro™ technology in the dsDNA application for samples within a certain concentration range. For detection of protein and phenol, dsDNA samples must be within 25 – 3,125 ng/µL to ensure accurate prediction by the Acclaro technology (see Table 2).

Application	Detected contaminants	Sample concentration
dsDNA	Protein, Phenol	0.5A – 62.5A 25 – 3,125 ng/µL
	Mammal, Bacteria, Plant RNA	1.0A – 20A 50 – 1,000 ng/µL
RNA	Protein, Phenol, Guanidine Isothiocyanate	0.5A – 62.5A 20 – 2,500 ng/µL
	Mammal, Bacteria, Plant DNA	1.0A – 20A 40 – 800 ng/µL
Protein A280	DNA	All concentrations

Table 2. Contaminants detected using the dsDNA, RNA, and Protein A280 applications with their associated sample concentration ranges.

Experimental procedures

Mixtures of dsDNA, protein, and phenol were created to yield the final concentrations as outlined in Table 3. The dsDNA was prepared by diluting Invitrogen™ UltraPure™ Calf Thymus DNA Solution (Invitrogen, 15633019). Protein was prepared from a bovine serum albumin solution (BSA, Sigma Aldrich, A7284), and phenol was prepared from a buffer-saturated stock (Fisher BioReagents, BP1750I). All mixtures were diluted in tris-EDTA (TE) buffer, pH 7.5, which served as the blank for the NanoDrop spectrophotometer measurements.

A Mixture #	1	2	3	4	5	6	7
Phenol (ppm)	1200	800	400	100	80	60	30
dsDNA (ng/µL)	250	250	250	250	250	250	250

B Mixture #	1	2	3	4	5	6
Protein (mg/mL)	10	4	2	0.75	0.5	0.25
dsDNA (ng/µL)	250	250	250	250	250	250

Table 3. A. Final concentrations of phenol and dsDNA mixtures. B. Final concentrations of protein and dsDNA mixtures.

A Thermo Scientific™ NanoDrop™ One^{C} Spectrophotometer served as the control instrument for comparing results generated from the NanoDrop Ultra instrument. For both instruments, fresh 2.0 µL measurements were performed using the microvolume pedestal in replicates of five with the dsDNA application, where the Acclaro Contaminant ID feature is automatically enabled for both models.

Results

The original and corrected dsDNA concentrations were automatically calculated in the experiment report. The average and standard deviation of each mixture were calculated outside of the software in addition to the average purity ratios (A260/A280 and A260/A230). The results are outlined in Table 4.

The Acclaro Contaminant ID feature was able to correctly identify the presence of contaminating protein in a dsDNA sample down to 0.5 mg/mL of protein or 2.5% (v/v) protein in the mixture. For phenol contamination, the Acclaro technology correctly identified phenol down to 60 ppm or 1.2% (v/v) phenol in the mixture. Both the protein and phenol sample sets displayed standard deviations of 5.5 ng/µL and below, indicating excellent reproducibility in calculating a corrected dsDNA concentration.

	Protein contamination						Phenol contamination							dsDNA control
Contaminant content	10 mg/mL	4 mg/mL	2 mg/mL	0.75 mg/mL	0.5 mg/mL	0.25 mg/mL	1200 ppm	800 ppm	400 ppm	100 ppm	80 ppm	60 ppm	30 ppm	-
Original dsDNA concentration (ng/µL)	460.0	345.4	294.6	280.8	264.9	267.5	702.1	563.3	401.0	303.8	292.1	285.0	271.9	254.8
Corrected dsDNA concentration (ng/µL)	293.1	267.7	240.0	259.3	250.2	-	278.7	274.3	256.3	264.5	274.9	259.2	-	-
Standard deviation of corrected dsDNA concentration (ng/µL)	1.1	5.5	0.7	0.5	0.9	0.7 (Original)	5.2	1.8	0.9	0.4	1.1	0.5	1.0 (Original)	0.4 (Original)
A260/A280 purity ratio	0.87	1.26	1.46	1.68	1.74	1.82	1.65	1.86	1.79	1.83	1.83	1.84	1.85	1.87
A260/A230 purity ratio	0.41	0.30	0.45	0.85	1.06	1.49	1.97	2.15	2.16	2.26	2.27	2.29	2.31	2.34
Acclaro Flag	⚠	⚠	⚠	⚠	⚠	No	⚠	⚠	⚠	⚠	⚠	⚠	No	No

Table 4. The original and corrected dsDNA concentrations from the NanoDrop Ultra instrument were averaged from five replicates. Standard deviation was reported for the corrected dsDNA concentration replicates unless marked as *Original* in the table, for which the standard deviation of the original dsDNA concentration was reported. The Acclaro Contaminant ID icon (⚠) indicates the software identified a sample contaminant.

Purity ratios for the phenol contamination samples were mostly within the acceptable range as detailed in Table 1. This demonstrates the importance of utilizing the Acclaro Contaminant ID feature for identifying contaminants that may not impact the purity ratios.

The percent error of the corrected dsDNA concentration from the NanoDrop Ultra instrument compared to the theoretical concentration for each sample is outlined in Figure 2. All corrected concentrations were within the 20% error limit of the Acclaro Contaminant ID technology, with the greatest error being 17.2% for the highest concentration of protein contamination (10 mg/mL). Most samples showed less than 10% error from the theoretical concentration, confirming the corrected dsDNA concentration accuracy.

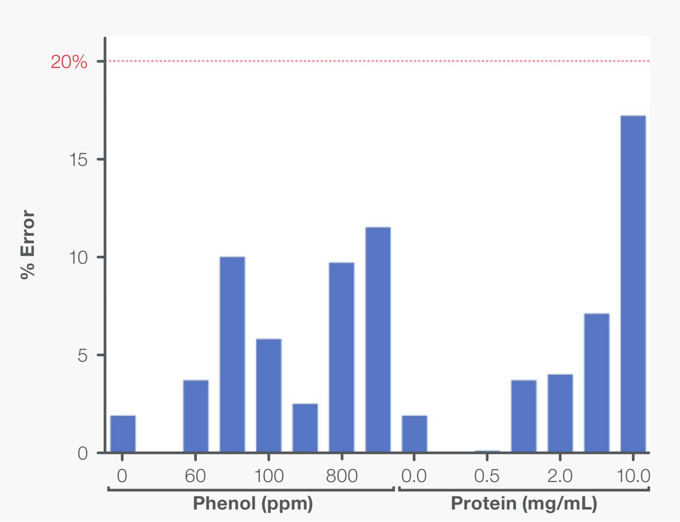

Figure 2. Percent error of the corrected dsDNA concentration from the NanoDrop Ultra instrument compared to the theoretical concentration. The red, dashed line denotes the 20% error limit of the Acclaro Contaminant ID feature. Bars are absent from the 30 ppm and 0.25 mg/mL samples as they were not flagged by Acclaro technology. Zero ppm and mg/mL represent the control dsDNA sample using the original concentration.

A comparison of the original, uncorrected concentrations and the Acclaro-corrected concentrations is outlined in Figure 3. As protein and phenol concentrations increased, uncorrected concentrations also increased, which highlights the impact of contamination on dsDNA concentration. After subtracting contaminants from the UV spectrum using the Acclaro technology, the corrected concentrations stayed within the ±20% error limit.

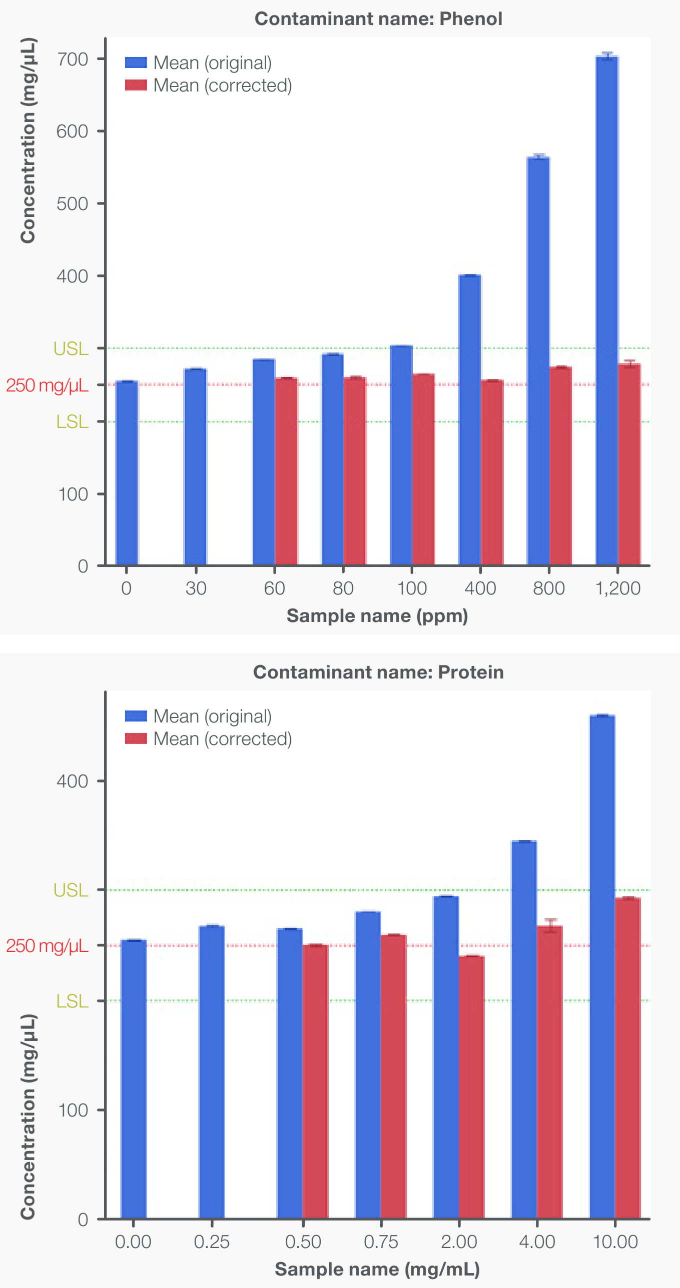

Figure 3. Bar graphs comparing the original, uncorrected dsDNA concentrations (blue bars) to the Acclaro-corrected concentrations (red bars) from the NanoDrop Ultra instrument. The red line represents the theoretical dsDNA concentration of 250 ng/µL and the green lines represent ± 20% error from the theoretical concentration to account for the Acclaro Contaminant ID algorithm error. Zero ppm and mg/mL represent the control dsDNA sample using the original concentration. A) Phenol contaminated samples. B) Protein contaminated samples. Error bars represent ± one standard deviation from the mean.

Figure 4 compares the Acclaro-corrected dsDNA concentrations from the NanoDrop One^{C} and NanoDrop Ultra spectrophotometers. For three samples, 60 ppm, 0.5 mg/mL, and 0.75 mg/mL, the NanoDrop Ultra software was able to calculate a corrected concentration while the NanoDrop One^{C} software was not. This sensitivity ensures low contaminant concentrations are accurately reported by the Acclaro technology on the NanoDrop Ultra instrument.

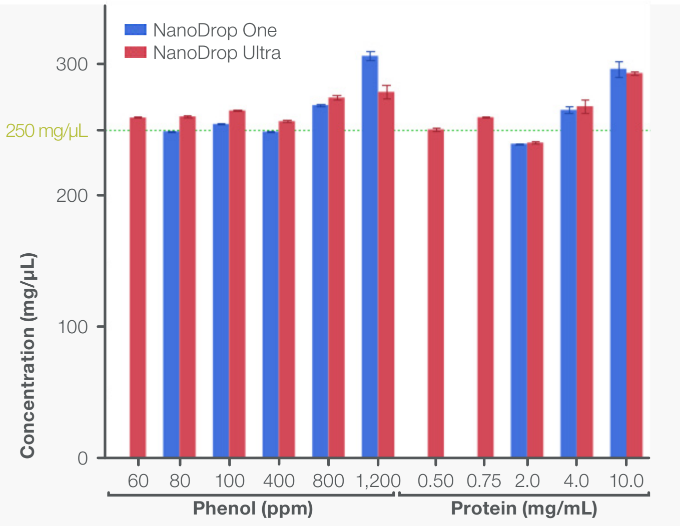

Figure 4. Comparison of Acclaro-corrected concentrations reported by the NanoDrop One^C (blue bars) and NanoDrop Ultra (red bars) spectrophotometers. The Acclaro technology on the NanoDrop One^C instrument did not flag a contaminant for samples without blue bars. The green line represents the theoretical concentration of 250 ng/µL. Error bars represent ± one standard deviation from the mean.

Conclusions

Historically, nucleic acid purity ratios provided the best indication of sample purity. Various contaminants, including salts, buffers, proteins, and polysaccharides, can affect purity ratios in ways similar to one another, making it challenging to identify and quantify a contaminant based solely on these ratios. To overcome this limitation, the Acclaro Sample Intelligence Contaminant ID technology utilizes chemometric algorithms and contaminant spectral libraries to analyze slight changes in the sample spectrum and provide predictions regarding the presence of contaminants. The NanoDrop Ultra spectrophotometer correctly identified protein and phenol contamination down to 2.5% (v/v) and 1.2% (v/v), respectively, with a mean corrected concentration percent error of 6.8% compared to theoretical. This feature offers crucial insights into purification protocols and the steps at which contaminants are typically introduced, allowing protocol adjustments that will prevent failed downstream reactions. The Acclaro technology built into the NanoDrop Ultra instrument removes the guesswork out of troubleshooting the presence of contaminants and is a useful tool for any life science laboratory.

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