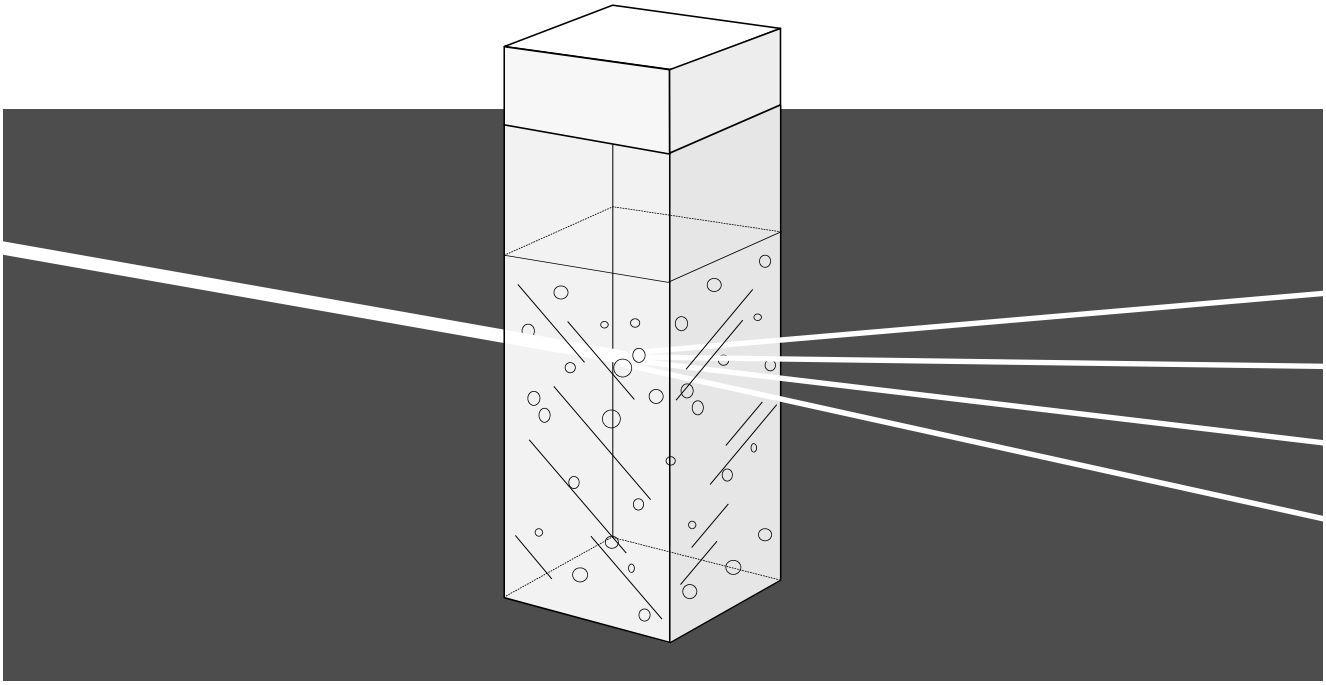
Quantitative UV-Visible Analysis in the Presence of Scattering

Application Note

Pharmaceutical Analysis

Anthony J. Owen



A common problem in the spectrophotometric analysis of pharmaceutical and biological samples is the presence of particles suspended in solution that cause scattering. In pharmaceutical samples these particles are usually the excipients or fillers used in tablet or capsule formulations. Scattering of radiation results in an apparent background *absorbance* that interferes with quantification. The problem of scattering can be eliminated by filtering samples before measurement but this may not always be practicable and the analyst must often work with spectra that include a scattering component. In this study various ways of correcting the measurements for the effect of scattering are compared using a computer-generated example.

Agilent Technologies
Innovating the HP Way

Introduction

The term *scattering*, as applied to the interaction of radiant energy with matter, covers a wide variety of phenomena. In the UV-Visible part of the spectrum there are two types of scattering:

– Rayleigh scattering, that occurs when the particles are small compared to the wavelength of the radiation, is inversely proportional to the fourth power of the wavelength, and

– Tyndall scattering, that occurs when the particles are larger, is inversely proportional to the square of the wavelength.

In chemical systems the exponent of the wavelength varies from -4 to -2 depending upon the distribution of particle sizes.

$$Absorbance_{scatter} \alpha \frac{1}{\lambda^n}$$

λ wavelength
n scattering order

Scattering causes an apparent absorbance because less light reaches the detector. Figure 1 shows typical spectra for Rayleigh and Tyndall scattering. True absorbance due to the presence of an analyte is superimposed on the scattering background. If no correction for the scattering is made, quantification of the analyte is erroneously high.

Experimental

Synthetic spectra were generated using a Microsoft® Excel version 3.0 spreadsheet and comprised:

- a Gaussian band representing the analyte and generated by the equation:

$$Absorbance = I * \exp\left(\frac{(\lambda - \lambda_{\max})^2}{2*(W/2)^2}\right)$$

λ wavelength
I maximum absorbance
λ_{max} wavelength at absorbance maximum
W natural bandwidth

– a scattering component generated by the equation:

$$Absorbance = I * 190^2\left(\frac{1}{\lambda^n}\right)$$

I maximum absorbance value at the minium wavelength of 190 nm
n scattering order

– a noise component generated by the equation:

$$Noise = \frac{N*(2*R-1)}{\sqrt{S}}$$

R random number between 0 and 1
N noise component for a single scan
S number of scans averaged to generate final spectrum

The noise component was added to simulate, as closely as possible, the data that would be created in real sample measurements.

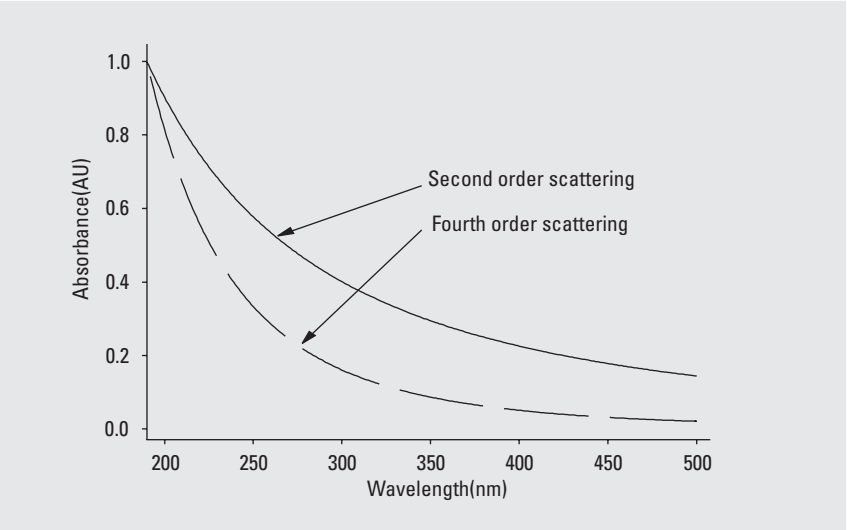


Figure 1
Apparent absorbance spectra caused by second and fourth order scattering

The data generated by the spreadsheet was saved as a .CSV text file. These files were processed using a Microsoft® Word macro to .WAV format that can be read by Agilent Technologies UV-Visible ChemStation software.

For this study the following spectra were generated:

- an analyte *standard* comprising a Gaussian band with λ_{max} at 300 nm, NBW of 20 nm, and maximum absorbance of 1 A,
- a scatter *standard* using fourth order scatter and an absorbance of 1 A at 190 nm, and
- five *sample* spectra comprising the Gaussian band analyte and the scatter component

Random noise of ±0.005 A per scan averaged over five scans was included in each spectrum. These values are typical of measurements made with an Agilent spectrophotometer. The random number calculator of the Microsoft® Excel spreadsheet makes sure that the noise component in all spectra is different. Figure 2 shows the synthetic spectra.

An Agilent UV-Visible ChemStation was used to perform all the quantitative calculations on the synthetic spectra including the correction techniques described in this note. The ChemStation comprised an HP Vectra 486/33N personal computer with Agilent G1115A general purpose and Agilent G1116A advanced UV-Visible ChemStation software runing under the Microsoft® Windows environment.

Correction Techniques

No correction

To create a reference, the five *sample* spectra were analyzed without any attempt to correct for scattering. The *standard* spectrum was used to perform a simple calibration at 300 nm using Beer's law. The results of quantification of the five samples are shown in column two of table 1. The average of the five samples shows an error of 16.09 % but the precision value of 0.011 % relative standard deviation (RSD) is excellent.

Internal reference

The simplest correction technique is to use a single reference wavelength. Generally it is best to choose a reference wavelength as close as possible to the analytical wavelength but where the analyte does not absorb significantly. The absorbance at the reference wavelength is subtracted from the absorbance at the analytical wavelength, correcting for any interference which is constant at all wavelengths as shown in figure 3. In the example an analytical wavelength of 300 nm and a reference wavelength of 380 nm were used.

The results of quantification are shown in column three of table 1. Accuracy has improved marginally to an error of 9.84 % and precision is still excellent at 0.011 % RSD.

The internal reference correction technique is available in all the Agilent UV-Visible ChemStation softwares.

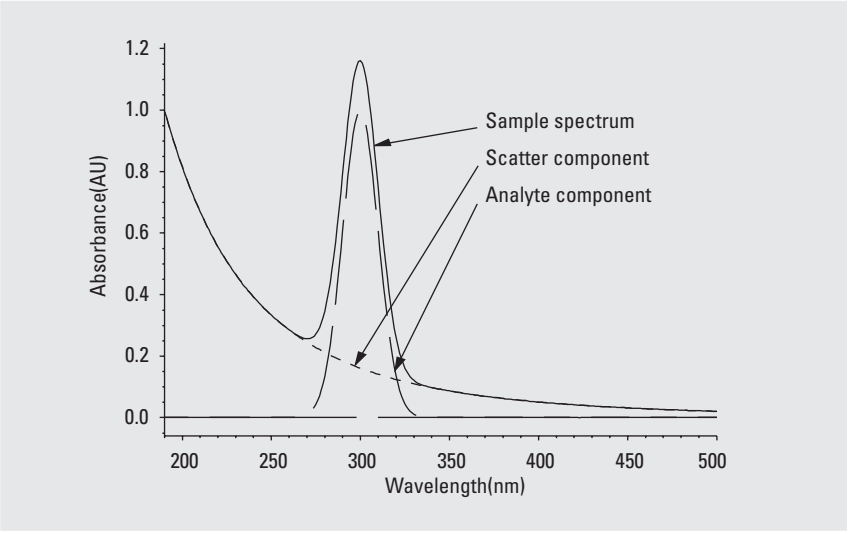


Figure 2
Synthetic scattering, analyte, and sample spectra

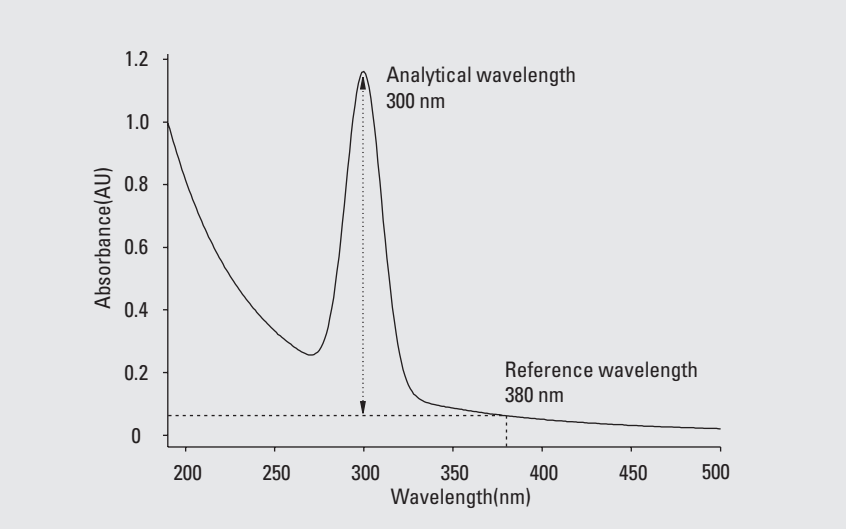


Figure 3
Internal reference correction

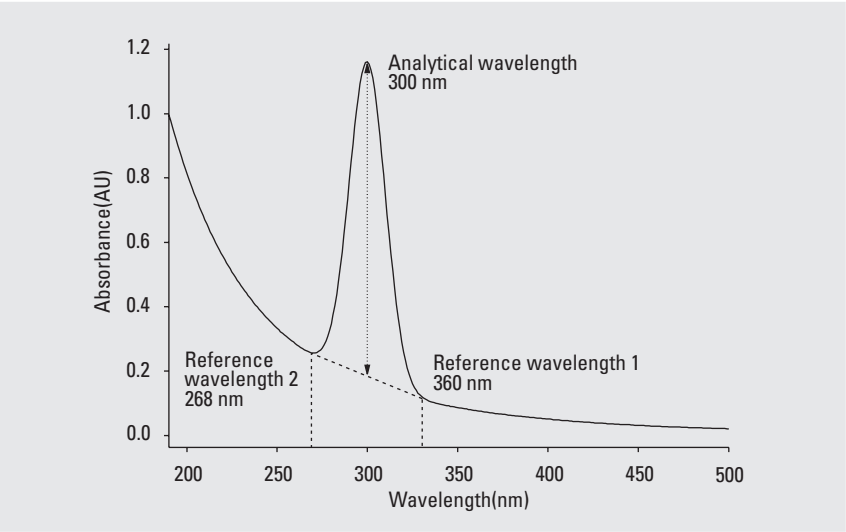


Figure 4
Three-point correction

Three-point

The three-point or Morton-Stubbs correction is probably the best known scatter-correction method. Two reference wavelengths are chosen either side of the analytical wavelength and the background absorbance at the analytical wavelength is estimated by linear interpolation as shown in figure 4. In the example 268 nm and 330 nm were selected as the reference wavelengths.

The results are shown in column four of table 1. The accuracy of 1.82 % is much better than with the internal reference calculation and the precision of 0.032 % RSD is also good.

The three-point correction technique is available in all the Agilent UV-Visible ChemStation softwares.

Derivatives

Derivative spectroscopy can discriminate between broad and narrow absorbance bands and is often used to minimize signal contributions from scatter. In our example we used both first and second derivative spectra. These were calculated using second-order polynomial and three-point smoothing (for more details of the use of derivative spectroscopy, see Agilent application note 5963-3940E).[1]

Figures 5 and 6 show first and second derivative spectra. To obtain the largest signal the difference between the absorbance at two wavelengths was used. For the first derivative we used 290 nm and 310 nm, and for the second derivative we used 282 nm and 300 nm.

The results are shown in columns five and six of table 1. The first derivative gives much better accuracy than the simple single wavelength calculation with an error of only -0.66 % but the precision of 0.13 % RSD is significantly worse. The second derivative shows very good accuracy with an error of -0.1 % but the precision of 1.26 % RSD is significantly worse. This worsening of precision is a consequence of the discrimination effect of derivatization. Noise has the narrowest bandwidth in the spectrum and therefore the signal-to-noise ratio decreases as higher derivatives are taken. This effect can be overcome by using a longer integration time.

The derivative correction technique is available in all the Agilent UV-Visible ChemStation softwares.

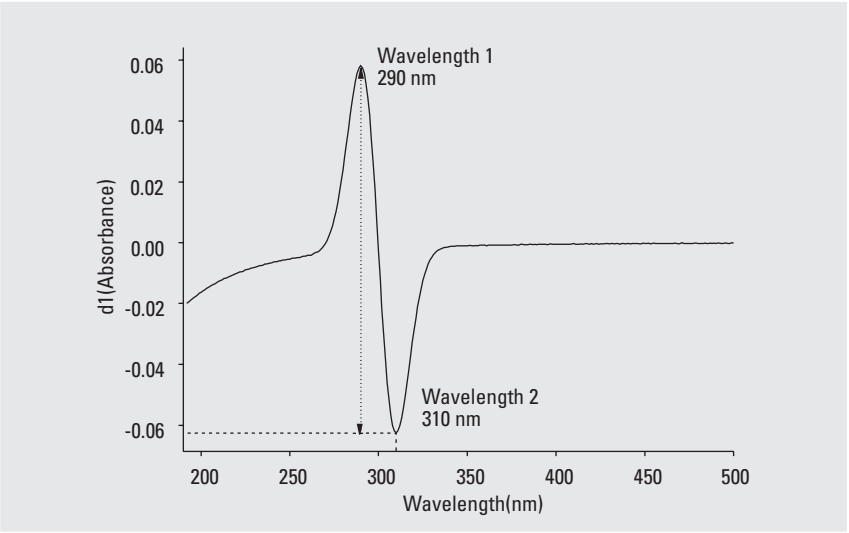


Figure 5
First derivative spectrum of sample

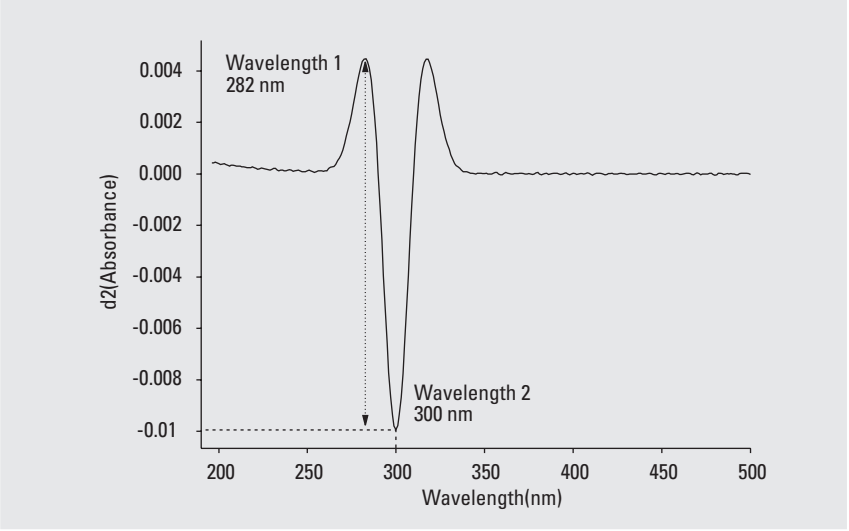


Figure 6
Second derivative spectrum of sample

Scatter modeling

In this method, a part of the spectrum is selected where the *absorbance* is caused only by the scatter. A polynomial is then fitted to this part of the spectrum using a least-squares fit to the logarithm of the absorbance.

$$A = a\lambda^n$$

$$\log(A) = \log(a) + n\log(\lambda)$$

Using the coefficients determined from the fit it is then possible to calculate the scatter contribution at all other wavelengths. These values are then subtracted from the measured values to leave the absorbances of the analyte. In our example we used the wavelength range 400 nm to 600 nm.

The results are shown in column seven of table 1. The accuracy is excellent with an error of only -0.04 % but the precision of 0.029 % RSD is only moderately good.

Note that this method will only work when:

- there is a part of the spectrum where absorbance is caused only by scattering,
- the scattering matches the model, and
- the background absorbance is caused only by scattering.

The scatter modeling correction technique is available in the Agilent G1116A advanced UV-Visible ChemStation software.

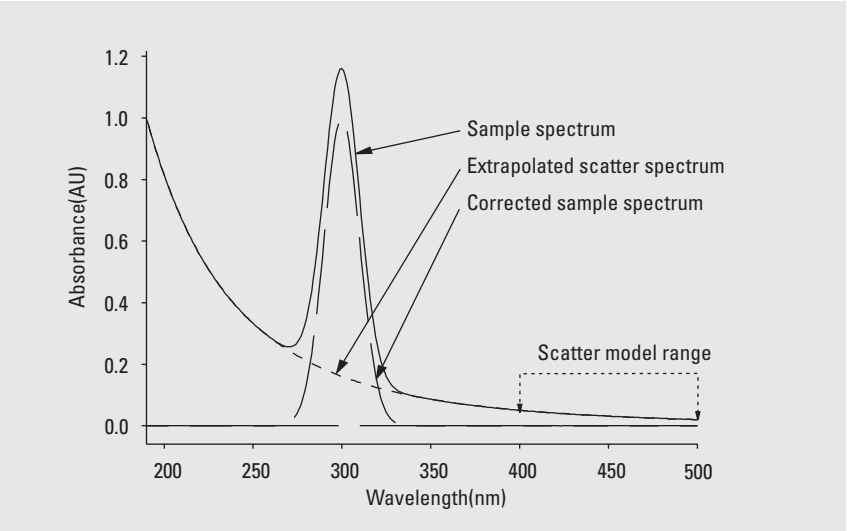


Figure 7
Scatter modeling

Multicomponent analysis

This method requires not only a spectrum of the analyte but also a spectrum of the scatter. When the component that causes the scattering is known (for example, the fillers in a tablet) a spectrum of the scatter can be obtained by making a suspension of the scattering component in solvent and measuring its spectrum. When the component is unknown, a spectrum can be obtained by filtering a sample, suspending the residue in solvent, and then measuring the spectrum. In this case the scatter spectrum was calculated as described above.

The multicomponent analysis was performed using the wavelength range 200 nm to 500 nm and a least-squares fit, see figure 8.

The results are shown in column eight of table 1. Both accuracy and precision are excellent with values of 0.002 % error and 0.005 % RSD respectively. However, this method is highly dependent on having an accurate scatter spectrum. If the order of scatter varies from sample to sample, this method will not work well.

The multicoponent analysis correction technique is available in the Agilent G1116A advanced UV-Visible ChemStation software.

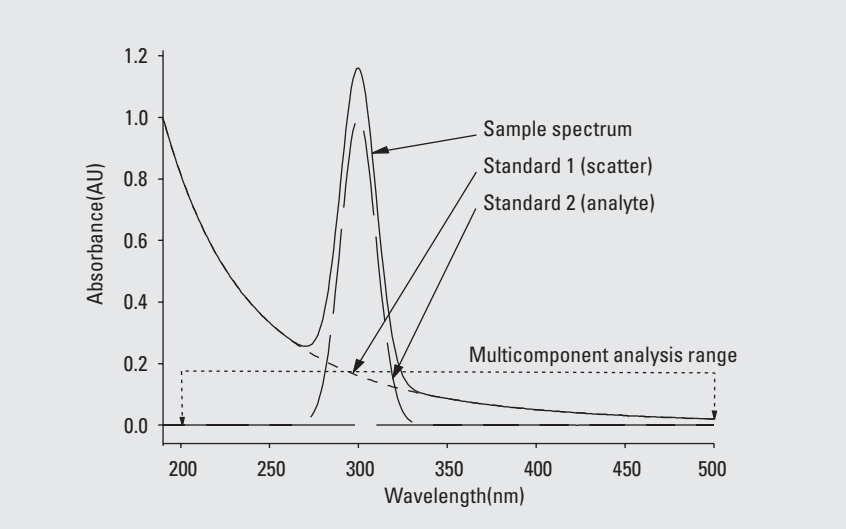


Figure 8
Multicomponent analysis

Sample	Simple 300 nm	Reference 300-380 nm	Three-point 300-(268,330) nm	First derivative 290-310 nm	Second derivative 282-300 nm	Scatter corrected 300 nm	Multicomponent 200 to 500 nm
1	1.16081	1.09838	0.98127	0.99400	0.99179	0.99986	0.99997
2	1.16080	1.09849	0.98158	0.99177	0.99915	0.99995	0.99991
3	1.16096	1.09822	0.98183	0.99233	0.99505	1.00018	1.00002
4	1.16111	1.09847	0.98202	0.99478	1.02041	1.00027	0.99996
5	1.16084	1.09827	0.98175	0.99418	0.98853	0.99954	1.00002
Average	1.160904	1.098364	0.98178	0.99341	0.998986	0.999961	0.999977
%Error	16.09 %	9.84 %	-1.82 %	-0.66 %	-0.10 %	-0.004 %	-0.002 %
RSD	0.00013	0.00012	0.00016	0.00130	0.0126	0.00029	0.00005
%RSD	0.011 %	0.011 %	0.016 %	0.13 %	1.26 %	0.029 %	0.005%

Table 1
Quantification results

Summary

A wide range of techniques are available to correct analytical measurements for the effects of scatter. This study shows that the best correction techniques (derivative, scatter modeling, and multicomponent analysis) require spectral data and that the derivative technique requires an excellent signal-to-noise ratio.

Diode-array spectrophotometers, with fast spectral measurement and low noise characteristics, fulfill these requirements. The Agilent G1115A general purpose and Agilent G1116A advanced UV-Visible ChemStation software allow theses techniques to be used easily in both method development and routine lab environments.

Note that some of the techniques described here (for example, three-point with second order derivative or multicomponent of derivative spectra) may be combined to give better results in more difficult situations.

References

1
Anthony J. Owen,
Agilent Technologies Application Note, **1994**, publication number 5963-3940E

Anthony J. Owen is a product manager based at Agilent Technologies, Waldbronn, Germany

Publication Number 5963-3937E

Agilent Technologies
Innovating the HP Way