



Abstract

HORIBA's Veloci™ Wine Analyzer is uniquely equipped with patented simultaneous Absorbance-Transmittance and excitation-emission matrix (EEM) technology (A-TEEM™) which provides rapid access to a wide range of parameters important to commercial wine processing and quality characterization. The Veloci Wine Analyzer acquires a complete UV-Vis spectrum including the industry standard Absorbance wavelength values at 280, 420, 520 and 620 nm which are important to evaluate a wine's phenolic content, Hue and Intensity. The Veloci Wine Analyzer also reports the Transmission spectrum which can be used to determine the CIE Lab Tri-Coordinate Color Descriptions. Most importantly, the Veloci Wine Analyzer reports a NIST-traceable EEM which can be evaluated using multivariate statistics. EEM analysis fingerprints yield qualitative and quantitative composition of key flavor and color determinants that are not discernible with simple Absorbance or Transmittance data analysis. Together the Absorbance, Transmittance and EEM data can be used to evaluate lot-to-lot, regional, and varietal characteristics, as well as sense the effects of oxidation and sulfite treatment, thus making the Veloci Wine Analyzer a valuable tool for industrial wine characterization.

Introduction

Red wines contain several colored and fluorescent components, mostly polyphenolic in nature, which determine various quality parameters, including color and flavor. Fluorescence and UV-VIS spectroscopy have the potential to detect and resolve these components to effectively characterize unique compositional properties of red wines from different sources and global regions and as a function of aging, processing and oxidation.

This study employed the patented A-TEEM method of simultaneous Absorbance and fluorescence excitation-emission matrix (EEM) spectroscopy on the Veloci Wine Analyzer. The main goal of this study was to investigate and document the expected synergistic information gained by simultaneous Absorbance, Transmittance and EEM analysis with respect to statistically significant resolution of wine samples in terms of color and their composition. The Absorbance and Transmittance spectra were evaluated

with respect to key wavelength parameters and CIE color index information commonly used by the wine industry for process evaluation and tolerance settings in quality control programs. The EEM data were analyzed using multivariate chemometric analyses including Parallel Factor Analysis (PARAFAC) and Principal Components Analysis (PCA). PARAFAC and PCA were used to determine their effectiveness to resolve both spectral and concentration information of the colored wine components. The results were discussed in light of their significance for lot-to-lot, varietal, developmental and or any other type of wine process characterization based on color composition analysis.

Materials and Methods

Seven types of the following red wines were prepared for the analysis. Each wine production area and the grape cultivar % are described in Table 1.

The samples were analyzed at room temperature and diluted with deionized water in a 1-cm path quartz fluorescence cell for an adjusted optical density (OD) of

Table 1. Wine varieties and grape cultivar compositions used in this study.

Production Region	Grape Cultivar
Italy (Italy)	50% Cabernet Sauvignon 50% Merlot
Italy (Italy N/A)	ND
Chile	Merlot
California (CA)	Merlot
Argentina (Arg)	Malbec
France (France)	55% Cabernet Sauvignon 40% Merlot 5% Petit Verdot
Spain (Spain)	50% Tempranillo 50% Gamacha

0.6 cm⁻¹ at 278 nm. Each sample's EEM and Absorbance spectrum were measured in triplicate. The samples were measured both when the bottle was freshly opened (within 1 hour) and after one week exposure to air.

The Veloci Wine Analyzer EEM and Absorbance measuring conditions included an Excitation/Absorbance range from 200-800 nm with a 3 nm increment and an emission range of 250-800 nm with a 4.65 nm CCD bin increment at medium gain and 0.1 s integration. All EEMs were normalized based on water Raman scattering units for the defined emission conditions. NIST-traceable spectral EEMs were corrected for the influence of Inner Filter Effects (IFE) and Rayleigh masking prior to PARAFAC and PCA analysis.

Results and Discussion

Typical EEMs, Absorbance and Percent Transmission Curves for Red Wine

Figure 1 shows simultaneously recorded EEMs (A and B) and Absorbance and percent Transmission spectra (C and D) for a typical Italian red wine from a freshly opened bottle Before (A and C) and After a one week exposure to air (B and D). The complexity of the EEM spectral contours, which comprise multiple overlapping excitation and emission components, limits qualitative and quantitative visual interpretation to major contour elements. Hence, multivariate analysis is generally applied to decompose the EEM matrix in terms of quality and quantity as will be described later. The complex EEMs however clearly exhibit the major contours in the UV excitation-emission range with the major excitation/emission (Ex/Em) peak around 275/309 nm. For both the Before and After samples the Absorbance (and Transmittance) spectra exhibited a major extinction peak around 275 nm, a smaller shoulder peak around 320 nm and a second minor peak around 520 nm. The 275 nm peak region is commonly associated, at least in part, with phenolic compounds and the 520 nm peak region is generally associated with anthocyanin compounds. Compared to the Before sample (C) the After samples (D) exhibited increased extinction across the entire Absorbance spectrum associated with the oxidation phenomenon. Likewise, the After EEM (B) showed stronger emission Intensity than the Before EEM (A) for all spectral contour features.

Absorbance, Transmittance and CIE Lab Analysis

The wine industry has adopted a conventional analysis involving three discrete Absorbance wavelengths, namely A420, A520 and A620 nm to rapidly establish basic color characteristics of wine, including the wine's redness, brownness or yellowness, Hue and Intensity. The Veloci Wine Analyzer's complete UV-VIS spectrum provides access to these wavelength parameters to facilitate these analyses. The Hue parameter is calculated as:

$$\text{Hue} = A420/A520$$

and the Intensity is calculated as:

$$\text{Intensity} = (A420 + A520 + A620).$$

Noting as mentioned above that the A280 value provides a metric for the presence of phenolics which may, however, also include Absorbance from many other compounds in this wavelength region.

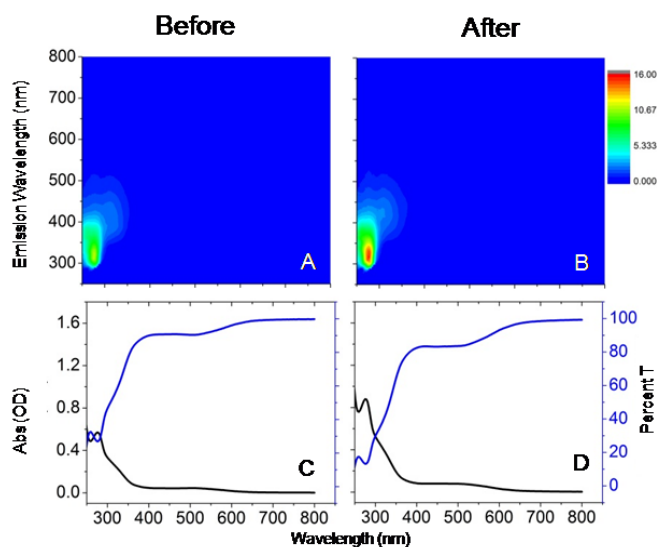


Figure 1. Fluorescence EEMs (A and B) and corresponding (C and D) Absorbance (OD) and %Transmittance (Percent T) spectra for the Italy wine sample Before (A and C) and After (B and D) a one week oxidation treatment.

Figure 2 compares the Absorbance parameters (A) and Hue and Intensity parameters (B) defined above, measured with the Veloci Wine Analyzer for a series of freshly opened red wines from various countries. The samples in Figs. 2A and B were arranged from left to right according to the decreasing average Intensity in Panel B. The Italy N/A sample showed the highest Intensity value and the France sample showed the lowest. The Intensity parameters A420, A520 and A620 in Panel A did not necessarily correlate with the A280 values which always exhibited the highest Absorbance peak in each wine. Likewise the Hue parameters, which ranged from around 0.82 to 1.07, more or less randomly among the samples in Panel B, did not correlate strongly with the Intensity parameters.

Table 2 reports the Absorbance, Hue, Intensity and CIE Lab color index parameters for the Italy wine sample shown in Figure 1 Before and After oxidation. Consistent with the EEM, Absorbance and Transmittance data in Figure 1, the

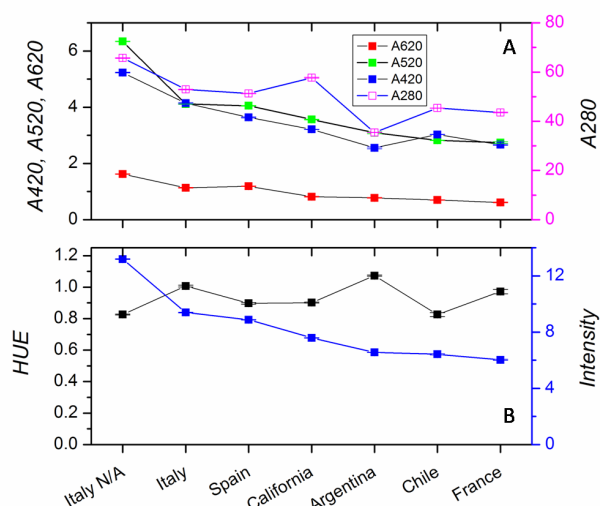


Figure 2. Comparison of the Absorbance parameters (A) and the Hue and Intensity parameters (B) for freshly opened wine samples (n=3 replicates per sample) from various locations. All Absorbance parameters (cm⁻¹) were adjusted for the sample dilution factor.

Table 2. Absorbance and CIE Lab color parameters compared before and after oxidation for the Italy wine sample (n=3 replicates per sample). Absorbance parameters were adjusted for dilution factor (96.15 fold) whereas CIE Lab Coordinates were calculated from the diluted samples.

Absorbance	Before	σ	After	σ	Δ	σ
A620	1.135E+00	1.091E-02	2.113E+00	1.170E-02	-9.780E-01	1.600E-02
A520	4.116E+00	1.502E-02	7.148E+00	1.704E-02	-3.032E+00	2.271E-02
A420	4.142E+00	1.858E-02	7.600E+00	1.136E-02	-3.458E+00	2.178E-02
A280	5.296E+01	1.378E-01	8.213E+01	1.355E-01	-2.917E+01	1.933E-01
HUE	1.006E+00	5.819E-03	1.063E+00	2.992E-03	-5.689E-02	6.543E-03
Intensity	9.392E+00	2.627E-02	1.686E+01	2.359E-02	-7.468E+00	3.530E-02

Before – After= Δ samples were negative indicating a significant ($p < 0.05$) increase in all Absorbance parameters and hence the Intensity parameter. The Hue parameter also increased significantly. It follows that all of the CIE Lab color indices registered significant changes ($p < 0.05$) associated with the oxidation treatment.

EEM PARAFAC Analysis

PARAFAC is a three-way analysis technique yielding an excitation and emission spectral shape loading and concentration score for each assumed model component. PARAFAC results are evaluated by a least-squares fitting figure of merit (r^2), in addition to residuals analysis, core-consistency and split-half validation tests to evaluate the model fit and parameter redundancy. The PARAFAC model evaluated in this study was constrained to yield non-negative values for all loading and score parameters. Figure 3 shows the excitation-emission contours for the five spectral loading components resolved in the PARAFAC model developed using all the fresh and oxidized wine sample replicates. Component 1 exhibited one major Ex/Em contour (275/330 nm) and a minor contour (275/425 nm). Component 2 exhibited one contour (260/370 nm). Component 3 showed the deepest UV emission with a major contour (275/300 nm) and a very minor contour (275/370 nm). Component 4 was broad in emission and bimodal in excitation with the major contour (325/410 nm); the deeper UV excitation band may not have been fully resolved above the 250 nm scale used in the analysis. Component 5 was present at low levels in all samples with a broad contour (430/560 nm). Based on the resolved Ex/Em coordinates, the components were compared to literature values for tentative identification as shown in Table 3 (Airado-Rodríguez et al.).

Figure 5 compares the effects of oxidation on each of the PARAFAC components for the Italy wine sample. Component 1 was the dominant component before and after oxidation. While all five components increased significantly ($p < 0.05$) in Intensity loading after oxidation, the deeper UV emitting components 1-3 increased relatively more than the longer emission wavelength components 4 and 5.

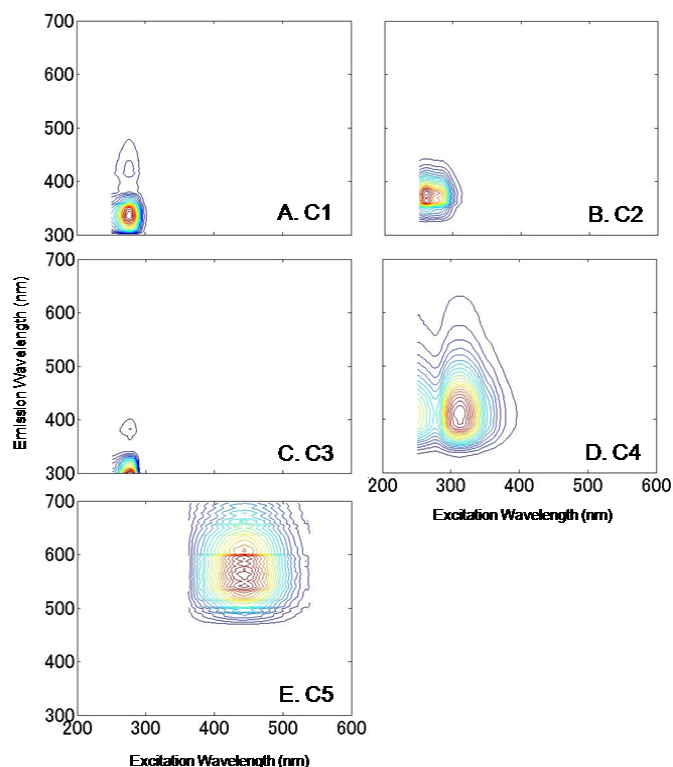


Figure 3. PARAFAC excitation and emission spectral contour loadings for the five component model evaluated for all wine sample replicates before and after oxidation. The model ($n=84$) was described with an $r^2=997$ and a split half validation score $r^2=0.906$.

Table 3. Qualitative assignment of PARAFAC components.

PARAFAC Component	Excitation Max, nm	Emission Max, nm	Name
C1	278	340	Caffeic Acid
C2	263	380	Flavonol Like
C3	280	300	Epicatechin
C4	315	405	Gentisic Acid
C5	445	568	Anthocyanin

Varietal Fingerprint-Cluster Analyses with PARAFAC and PCA

The three major fluorescence PARAFAC and two-way Principal Components Analysis (PCA) model component loadings were respectively evaluated in Figure 6 A and B for all the fresh wine samples by cluster analysis. PCA is a two way analysis technique that yields component scores that can show negative amplitudes and thus may be considered physically unrealistic in terms of chemical component spectra. PCA components however may still be indicative of qualitative and quantitative changes in the sample's spectral composition.

Discussion and Conclusion

The data clearly indicate the Veloci Wine Analyzer's patented A-TEEM simultaneous Absorbance and EEM acquisition and analysis, as described in Figure 6, can uniquely provide significant resolution of wine varieties and treatments based on the basic Absorbance parameters such as Hue and Intensity and the CIE Lab parameters derived from the Transmission data. Notably the information provided by the Absorbance and Transmittance analyses represents the aggregate effects of all overlapping spectral components contributing to the processed signals. Therefore it is important to note the EEM analyses provide both valuable qualitative and quantitative information on individually resolved color components. Clearly all the analyses are of potential value to industrial wine evaluation and can be extended to a wide variety of applications beyond those described in this study.

References:

Steve Upstone, Wine Analysis Using the LAMBDA Series Spectrophotometers, PerkinElmer, Inc.
Diego Airada-Rodriguez, Isabel Duran-Meras, Teresa Galeno-Diaz, Jens Petter Wold, Front-face fluorescence spectroscopy: A new tool for control in the wine industry, Journal of Food Composition and Analysis 24 (2011) 257-264

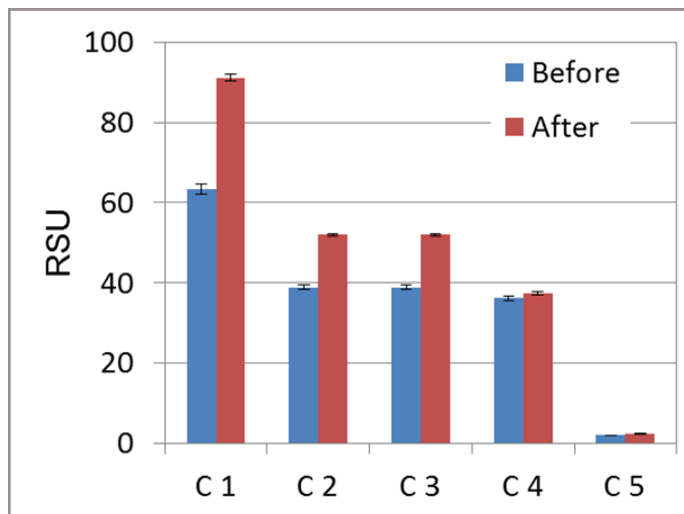


Figure 4. Comparison of the five PARAFAC component scores in the Italy wine samples (n= 3 replicates per sample) before and after oxidation. Component scores are reported normalized water Raman scattering units (RSU).

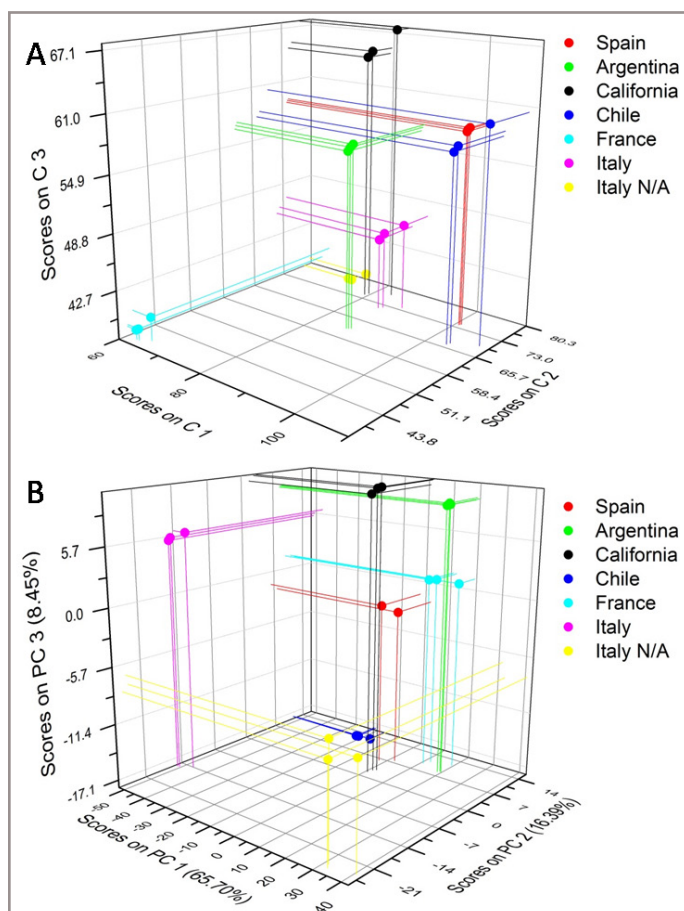


Figure 5. Comparison of the three major component cluster plots for PARAFAC (A) and PCA (B) analyses of the freshly opened wine samples (n=3 replicates per sample). All component scores are reported normalized to water Raman Scattering Units (RSU).

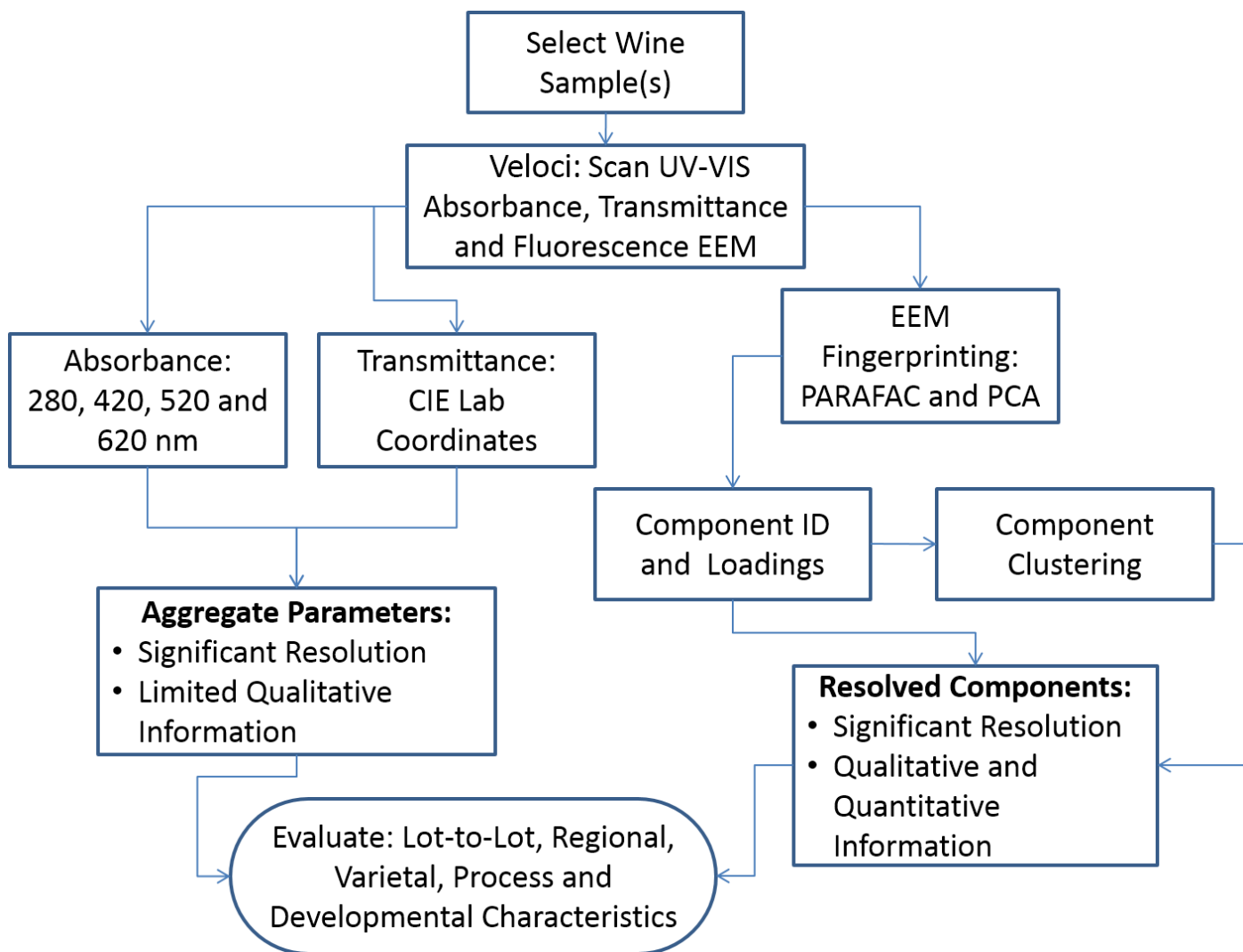


Figure 6. Schematic of the relationships among the simultaneous Absorbance and EEM (A-TEEM) data acquisition and analysis with respect to the significance of wine sample and component resolution pertaining to this study.

Glossary of Terms:

X, Y, Z: These are the tristimulus values and X, Y and Z for red, green and blue respectively.

x, y: These are two of the three normalized values which are functions of all three tristimulus values X, Y and Z.

L*: Lightness in the L*a*b* system. This system is basically a way of representing color in three dimensional space using Cartesian coordinates.

a*, b*: The color-component dimensions in the L*a*b* system.

C*: Chroma (C) is the radial component in the L*a*b* system.

h*: Hue angle (h) is the angular component in the L*a*b* system.

S*, Q*: The wine industry also uses these values to produce some additional parameters based on the L*a*b* and C*ab values by the following formulae.

$$S^* = C^*ab / L^*$$

$$Q^* = (0.15 \cdot L^*) \cdot \log(Yn) + (0.6 \cdot L^*) + 40$$

A-TEEM is a patented trademark of HORIBA Instruments Inc.

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