

# Velocity-corrected area calculation: SCIEX PA 800 Plus Empower Driver version 1.3 vs. 32 Karat™ Software

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Since the introduction of commercial capillary electrophoresis (CE) systems over 30 years ago, it has been important to not always use conventional “chromatography thinking” when using CE. This is especially true when processing data, as there are some key differences between electrophoretic and chromatographic data.

For instance, in most capillary electrophoresis separations, peak area is not only a function of sample load, but also of an analyte’s velocity past the detection window. In this case, early migrating peaks move past the detection window faster than later migrating peaks. This creates a peak area bias, as any relative difference in an analyte’s migration velocity will lead to an error in peak area determination and relative peak area percent. To help minimize this bias, peak areas are normalized by migration velocity. The resulting parameter is commonly referred to as corrected peak area or velocity corrected area.

This technical note provides a comparison of velocity corrected area calculations using 32 Karat™ and Empower software. For both, standard processing methods without manual integration were used to process each result. For 32 Karat™ software, Caesar integration<sup>1</sup> was turned off. For Empower software, both ApexTrack and Traditional processing methods are used.

## Key Features

- Calculation of velocity-corrected area (VCA)
- Correlation of VCA between 32 Karat™ software and Empower software
- The average absolute and relative percent difference of % VCA between 32 Karat™ software and Empower software’s (Apex and Traditional) are comparable.

## Methods

### Instrument

All experiments were performed on a PA 800 Plus System (SCIEX, Brea, CA) equipped with a PDA detector. Data was collected using the PA 800 Plus Empower™ Driver v1.3. A 50 µm internal diameter bare-fused silica capillary (SCIEX, P/N 338451) was installed to give 30.2 cm total length (effective length 20 cm).



Figure 1: The PA 800 Plus Pharmaceutical Analysis System.

The capillary temperature was maintained at 25°C in all separations. The voltage was applied using reverse polarity.

The following methods were used with the SCIEX PA 800 Plus Empower™ Driver v1.3:

- IgG\_HR\_Conditioning: conditions the capillary
- IgG\_HR\_Separation: performs IgG separation
- IgG\_HR\_Shutdown: cleans the capillary at the end of a sequence for storage and turns off the UV lamp

### Chemicals and materials:

The IgG Purity/ Heterogeneity Assay Kit (P/N A10663) was obtained from SCIEX (Carlsbad, CA). 2-mercaptoethanol was purchased from Millipore-Sigma (Burlington, MA).

### Sample preparation

#### Prepare the IgG control standard (reduced)

1. The IgG control standard (~ 1mL) was aliquoted into ~10 vials of 95 µl each and stored at -20°C to avoid multiple freeze-thaw cycles.
2. Thaw one of the 95 µL aliquots of the IgG control standard at room temperature.
3. Add 2 µL of the 10 kDa internal standard to the IgG tube.

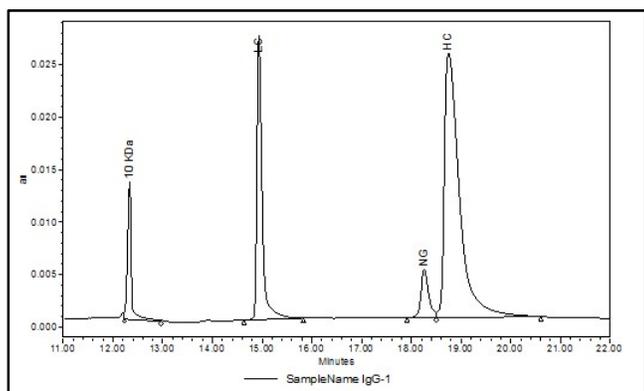
4. Inside a fume hood, add 5  $\mu$ L of 2-mercaptoethanol to the IgG tube.
5. Cap the tube and mix thoroughly.
6. Using a centrifuge, spin the tube for 1 minute at 300xg.
7. Seal the vial cap with Parafilm and then heat the vial at 70°C for 10 minutes.
8. Put the vial in a room-temperature water bath to cool for at least 3 minutes.
9. Transfer 100  $\mu$ L of the prepared sample to a micro vial, put the micro vial in a universal vial, and then cap the universal vial.

The data was collected on a SCIEX PA 800 Plus System using the SCIEX PA 800 Plus Empower Driver™ v1.3 and analyzed with the Empower software. The same data set was imported into 32 Karat™ software, analyzed and the results of the two analyses were compared.

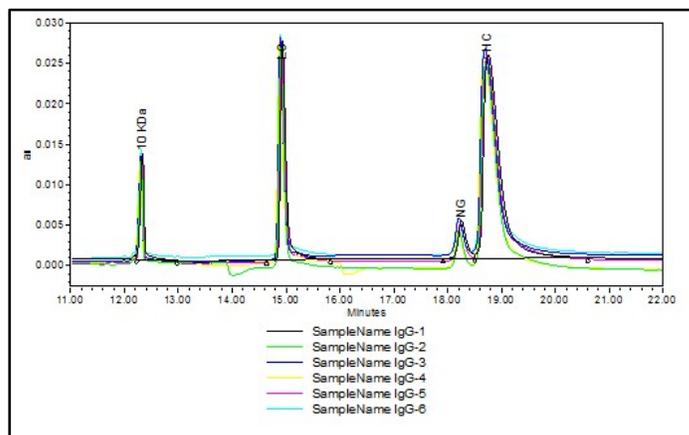
## Results and discussion

### Typical results for the reduced IgG control standard

The IgG control standard used in this assay includes a controlled percentage of non-glycosylated heavy chain, which provides both a resolution and a quantification benchmark<sup>2</sup>. Figure 2 shows one analysis of the reduced IgG control standard. Figure 3 shows an overlay of all six reduced IgG analyses used in the VCA analysis.



**Figure 2: Separation of the IgG control standard under reducing conditions**



**Figure 3: Overlay of six analyses of the IgG control standard under reducing conditions**

### Calculating velocity-corrected area

Velocity-corrected area is calculated using the following formula<sup>3</sup>.

$$A_{corr} = vA = \frac{L_d A}{t}$$

$A_{corr}$  = Velocity-corrected area

$v$  = Velocity

$A$  = Uncorrected peak area

$L_d$  = Capillary effective length (in cm)

$t$  = Migration time (in seconds)

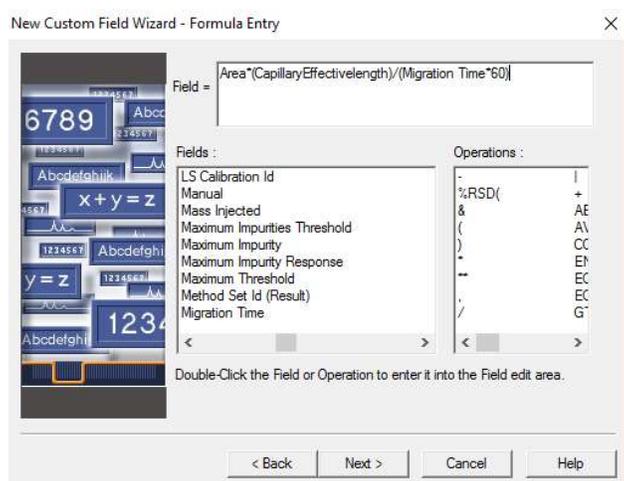
Several important features can be seen from this equation:

1. Velocities are measured terms. They are calculated by dividing the capillary effective length ( $L_d$ ) by the migration time of the analyte peak.
2. The capillary effective length,  $L_d$ , is important. The total length of the capillary ( $L_t$ ) is not used.
3. The migration time ( $t$ ) is the time it takes an analyte to move from the inlet of the capillary to the detection window.

Every type of chromatography data system software has its own method of integration and of calculating area. In the Empower software, VCA can be calculated either by entering the formula in a custom field using a fixed  $L_d$  value or by creating another custom field for capillary length. The resulting VCA value is the same regardless of how the calculation is performed. Tables 1, 2 and 3 show the reproducibility of the analysis of the reduced IgG samples using 32 Karat™ software and SCIEX PA 800 Plus Empower™ Driver v1.3.

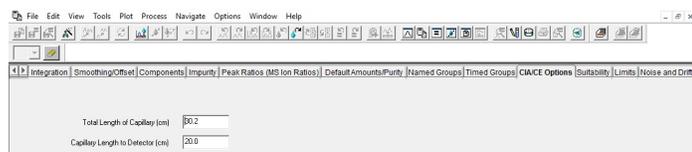
### Velocity-corrected area Custom Field

The formula for Velocity Corrected Area is entered as a Custom Field in Empower software as shown in Figure 4.



**Figure 4: Velocity-corrected area formula entry on Empower software**

Once the formula is entered as a Custom Field, the capillary length is entered in the processing method as shown in Figure 5.



**Figure 5: Capillary Effective Length entry in Empower Processing Method**

### Empower integration algorithms

The Empower software provides two integration algorithms to process data:

- Traditional: Uses the slope threshold (first derivative) to detect peaks.
- ApexTrack: Uses the peak apex as identified by the rate of change of the slope (second derivative) to detect peaks.

For VCA calculations using the Empower software, data was analyzed using both integration methods. Tables 1, 2 and 3 show the VCA calculation of six analyses of reduced IgG using 32 Karat™ software and the Empower software ApexTrack and Traditional integration algorithms.

**Table 1: % Reproducibility of % VCA for reduced IgG calculated using 32 Karat™ software.**

	Average	Std. Dev.	%RSD
% LC	32.0	0.079	0.25
% NG	6.7	0.057	0.86
% HC	61.3	0.065	0.11
% NG of HC	9.8	0.078	0.80

**Table 2: Reproducibility of % VCA for reduced IgG, calculated using Empower software (ApexTrack).**

	Average	Std. Dev.	%RSD
% LC	31.8	0.419	1.32
% NG	6.8	0.053	0.78
% HC	61.4	0.380	0.62
% NG of HC	9.9	0.050	0.50

**Table 3: Reproducibility of % VCA for reduced IgG calculated using Empower software (Traditional).**

	Average	Std. Dev.	%RSD
% LC	32.0	0.297	0.93
% NG	6.8	0.034	0.50
% HC	61.2	0.276	0.45
% NG of HC	10.0	0.039	0.39

Overall, the data is comparable between the two software programs and the two integration algorithms.

A comparison was also performed by calculating the relative percent difference between the average VCA generated with 32 Karat™ software and Empower software using either ApexTrack or Traditional integration algorithms (Table 4, 5).

**Table 4: Comparison of average absolute and relative percent difference of %VCA between 32 Karat™ software and Empower software ApexTrack integration**

	Absolute Difference	Relative Percent Difference
% LC	0.01	0.6
% NG	0.03	3.1
% HC	0.01	1.4
% NG of HC	0.01	1.5

**Table 5: Comparison of average absolute and relative percent difference of %VCA between 32 Karat™ software and Empower software Traditional integration**

	Absolute Difference	Relative Percent Difference
% LC	0.01	0.6
% NG	0.02	2.5
% HC	0.01	0.6
% NG of HC	0.01	1.7

The results show that data collected using the SCIEX PA 800 Plus Empower™ Driver v1.3 can be successfully used to calculate VCA.

## Conclusion

Overall, the VCA values generated using the Empower software are comparable to those generated using 32 Karat™ software. The data reported here are for comparison purpose only. Migration to the SCIEX PA 800 Plus Empower Driver can be done with confidence since the results from Empower software are comparable to those generated using the 32 Karat™ software.

## References

1. *Application of the Caesar Algorithm to Capillary Electrophoresis. T-1841A.*
2. *IgG Purity and Heterogeneity Assay Kit Application Guide RUO-IDV-05-6935-B*
3. *P/ACE MDQ User's Guide A33115AB*

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