Reconstruction of the out-of-range peaks using the Exponentially Modified Gaussian peak shape

Yuri Kalambet, Ampersand International, Inc., Beachwood, OH

Ksenie Mikhailova, Ampersand Ltd., Moscow, Russia;

Yuri P. Kozmin, Shemyakin Institute of Bioorganic Chemistry, Moscow, Russia;

Igor Nagaev, Institute of Molecular Genetics RAS, Moscow, Russia;

Pavel Tikhonov, The Russian Research Institute of Metrological Service, Moscow, Russia

e-mail: **kalambet@ampersand-intl.com**

www.chromandspec.com

Abstract

The Exponentially Modified Gaussian (EMG) peak shape [1] is widely used for peak approximation in chromatography. We constructed the EMG peak deconvolution routine for chromatography, using a combination of two EMG formulas [1,2] and linear optimization methods. This routine accounts for the maximum linear range of the detector and can work with out-of range peaks.

The optimization routine is applied to the reconstruction of out-of-range peaks, so that analyst can get an idea of the area/height and concentration of such peaks. We have found that in many cases such reconstruction provides a reasonable prediction error. This information helps in the reducing number of chromatographic runs during method development and routine work. The possibility of reconstructing out-of-range peaks using the pre-defined peak shape obtained while calibrating is also discussed.

Introduction

Peak reconstruction is done by the approximation of the peak by EMG, formula

$$F(t) = \frac{h \cdot \sigma}{\tau} \sqrt{\frac{\pi}{2}} \cdot e^{\left(\frac{\sigma^2}{2\tau^2} - \frac{t-\mu}{\tau} \right)} \cdot \left(1 - \mathbf{erf}\left(\frac{1}{\sqrt{2}} \left(\frac{\mu - t}{\sigma} + \frac{\sigma}{\tau} \right) \right)\right) \qquad 1)$$

where t is time, h – Gaussian height, σ – Gaussian sigma, μ – position of unmodified Gaussian, τ – relaxation time, parameter of exponent used to modify Gaussian and $\mathbf{erf}(z) = \frac{2}{\sqrt{\pi}} \int_0^z e^{-t^2} dt$

More precisely:

$$F(t) = \frac{h \cdot \sigma}{\tau} \sqrt{\frac{\pi}{2}} \cdot e^{\left(\frac{\mu - t}{\tau} + \frac{\sigma^2}{2\tau^2} \right)} \cdot \mathrm{erfc}\left(\frac{1}{\sqrt{2}} \left(\frac{\mu - t}{\sigma} + \frac{\sigma}{\tau} \right) \right) \qquad 2)$$

where $\mathbf{erfc}(z) = 1 - \mathbf{erf}(z)$.

Theory of Deconvolution

Alternative formula of EMG was derived by Deley [2] and in modern notations can be written as

$$F(t) = h \cdot e^{\frac{-(\mu - t)^2}{2\sigma^2}} \cdot \frac{\sigma}{\tau}\sqrt{\frac{\pi}{2}} \cdot \mathrm{erfcx}\left(\frac{1}{\sqrt{2}}\left(\frac{\mu - t}{\sigma} + \frac{\sigma}{\tau}\right)\right) \qquad 3)$$

where $\mathbf{erfcx}(z) = e^{z^2}\,\mathbf{erfc}(z)$. In the extreme case of very small τ/σ

$$F(t) \approx \frac{h \cdot e^{\frac{-(\mu - t)^2}{2\sigma^2}}}{\left(1 + \frac{(\mu - t)\cdot\tau}{\sigma^2}\right)} \qquad 4)$$

The Fourier transform procedure of EMG deconvolution cannot be used, as the Fourier spectrum of the overloaded peak is not suitable for deconvolution. Traditional optimization methods are used instead.

Materials and methods

Chromatograph (Bischoff Chromatography):

Detector – mod. Lambda 1010;

Pump – mod.2250, 0.01-4.99 ml/min pumphead;

Variotherm column thermostat, 35°C.

Separation:

Eluent: methanol-water-acetic acid (45:55:0.1), isocratic mode

Flow rate: 1 ml/min.

Column 1: Reprosil pur C18aq, 5um, 4*150 mm

Column 2: Kromasil 100C18, 6 мкм, 4*150 мм

Detection wavelength: 250 nm

Software: Chrom&Spec by Ampersand International, Inc.

Sample: nipagin (methyl n-hydroxybenzoat), solution 1mg/ml in methanol

Results

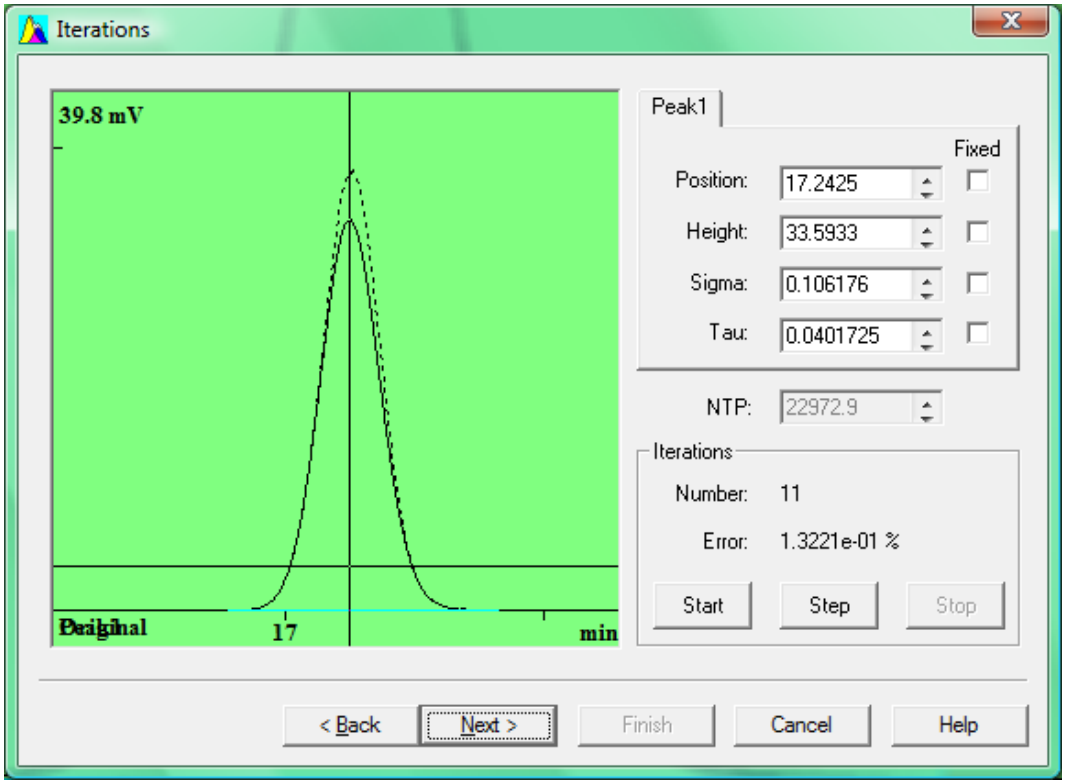

Figure 1. Window of the software deconvolution module. Reconstruction of the peak using 10% of the peak height. Original data are drawn by the dotted line, reconstructed – by the solid line. Only points between baseline and the horizontal line above the baseline are used for the reconstruction of the peak.

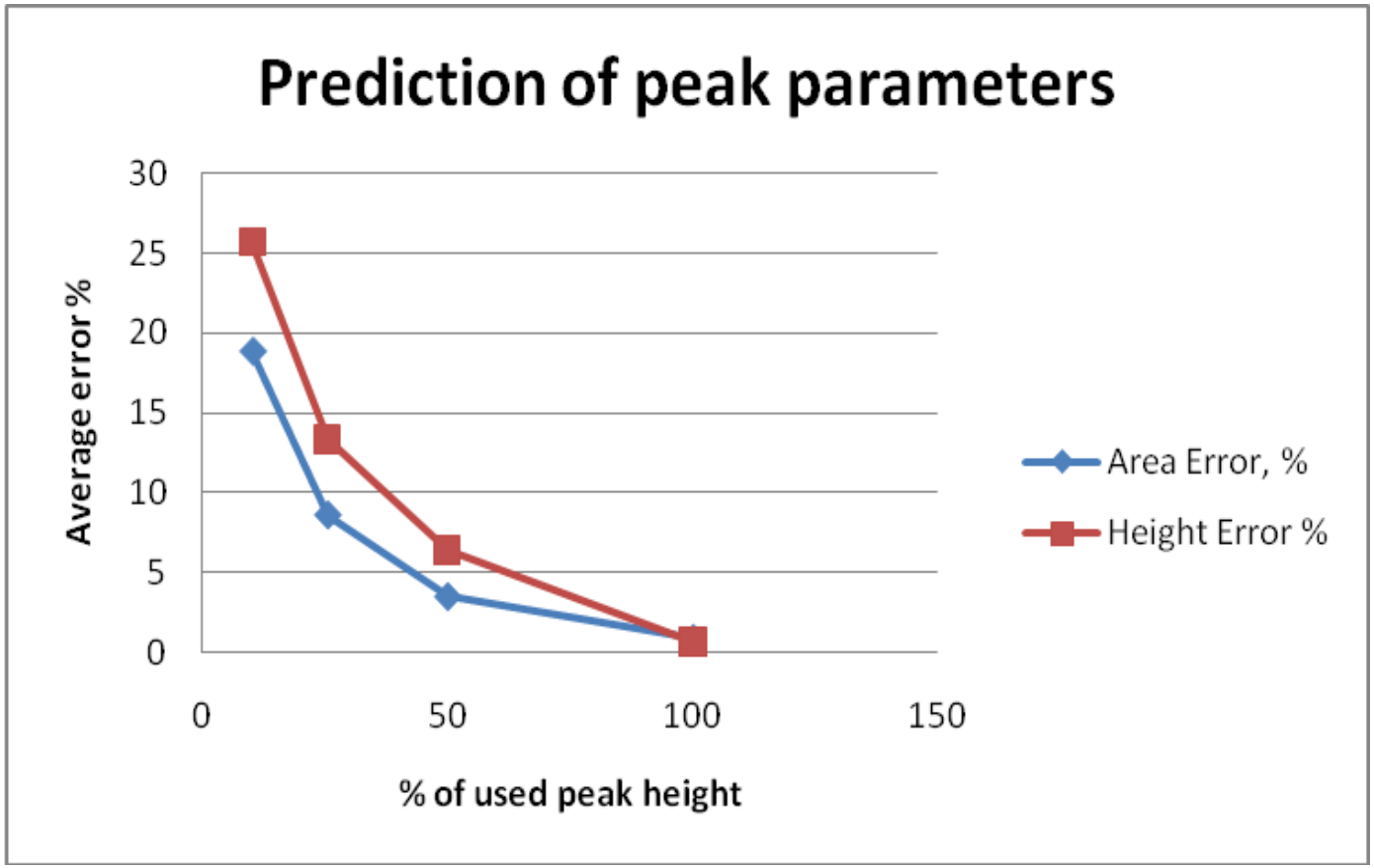

Figure 2. Prediction of the peak height and area for 44 stand-alone peaks from different chromatograms (all LC)

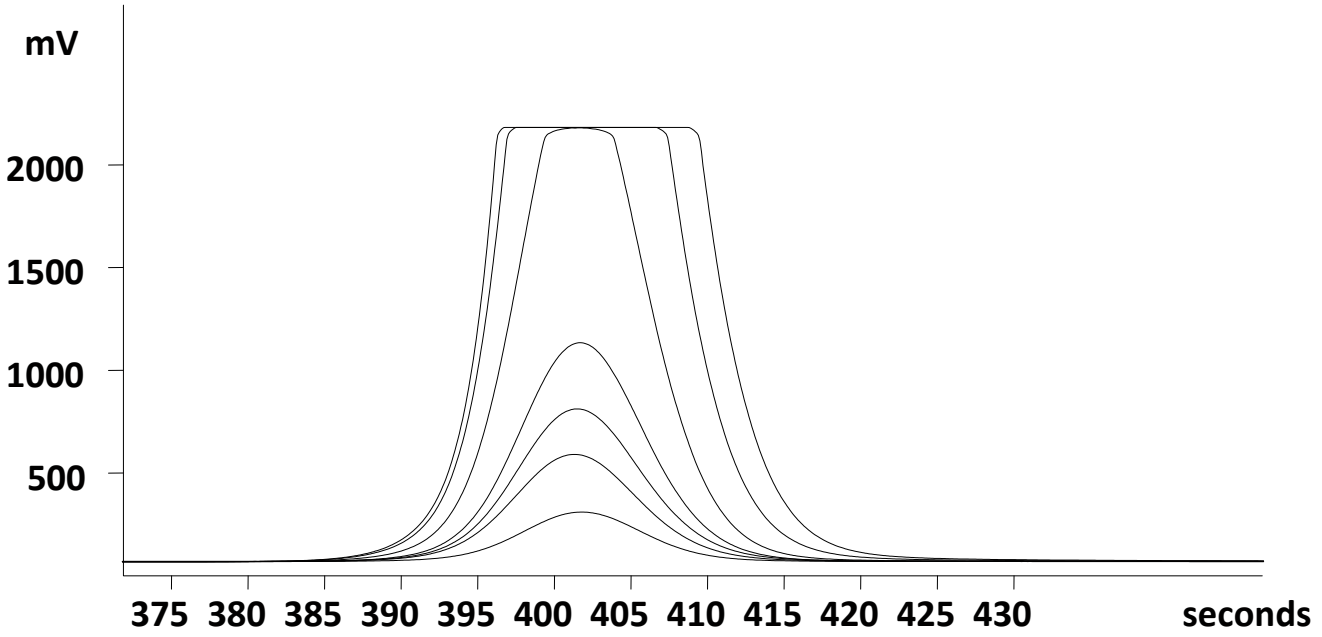

Figure 3. Series 1 of Nipagin chromatograms; concentrations 1: 2: 3: 4: 10: 20: 30

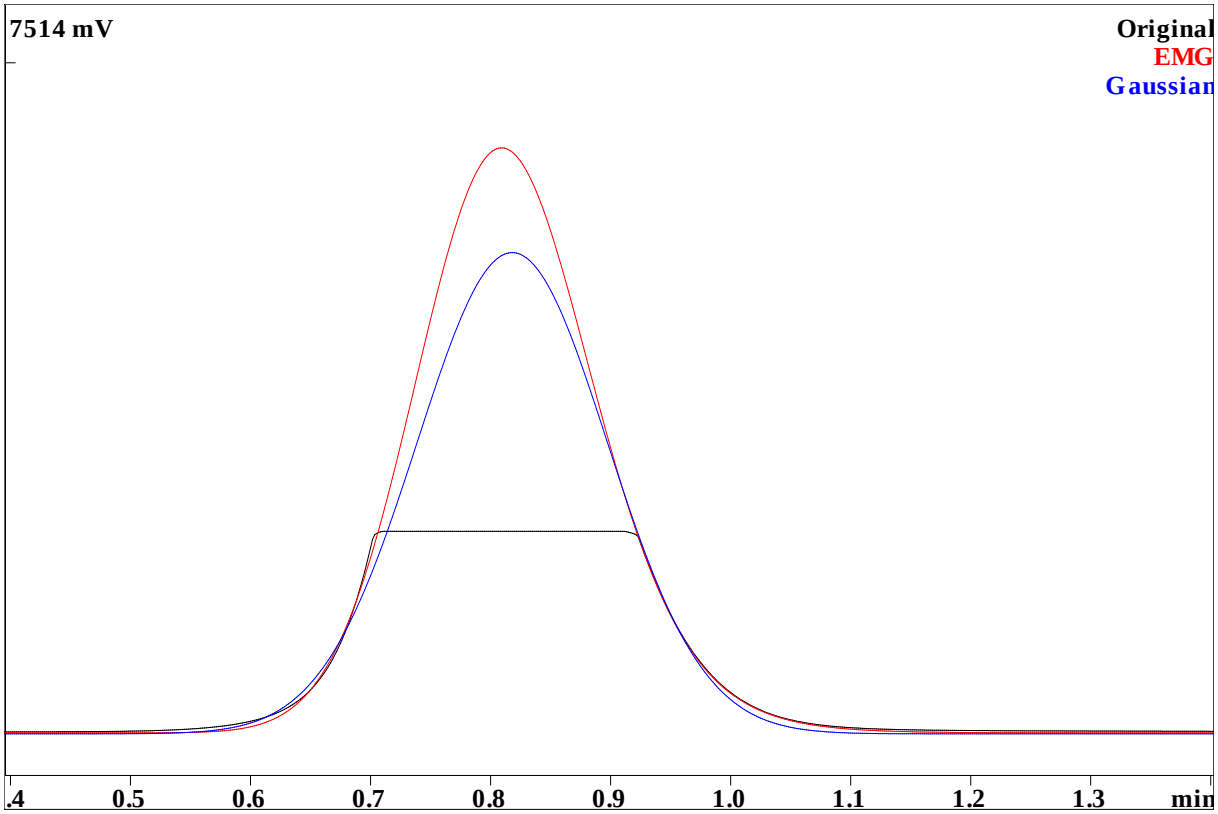

Figure 4. Reconstruction of the nipagin peak (black line) by EMG (red line) and Gaussian (blue line)

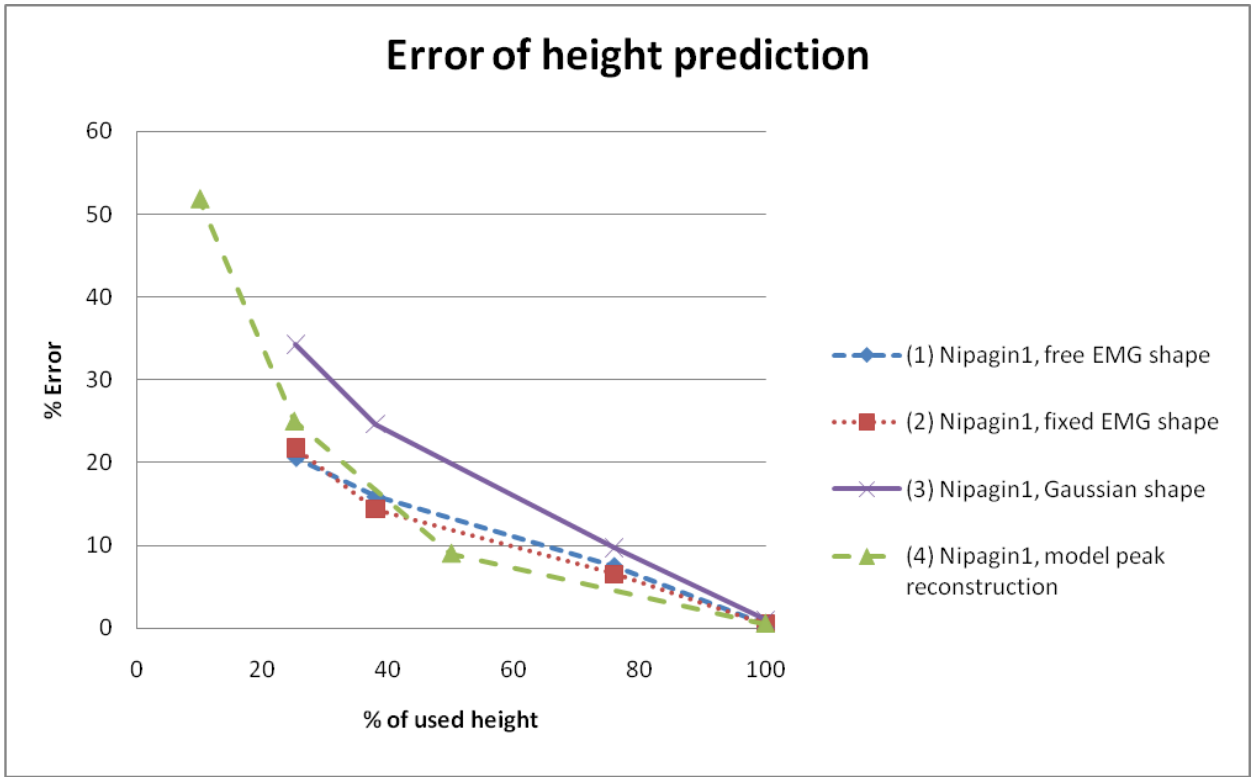

Figure 5. Peak reconstruction for Nipagin Series 1. For the fixed EMG shape σ and τ parameters of the EMG are fixed to the values, obtained for the Model peak; free EMG shape allow σ and τ to be optimized. The model peak is the highest "normal" peak (peak with concentration of 4 in Series 1).

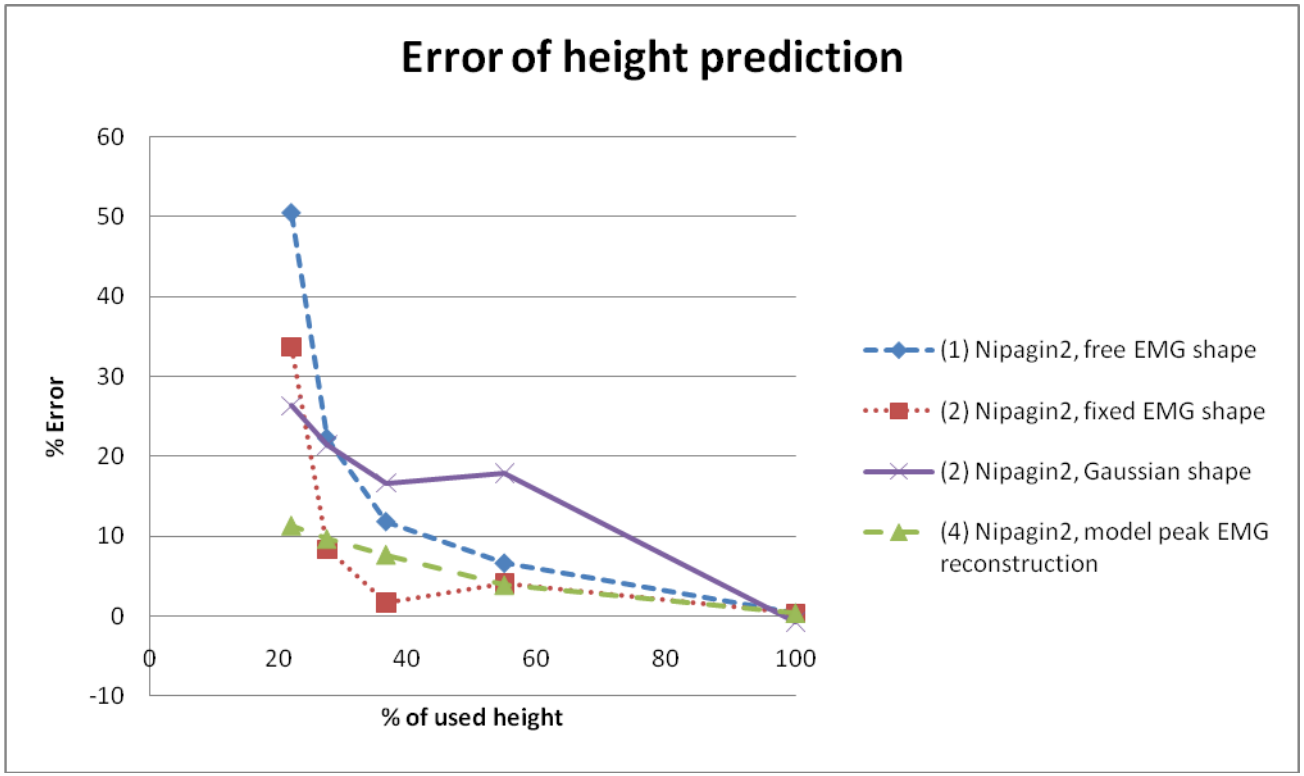

Figure 6. Peak reconstruction for Nipagin Series 2. Concentrations 1: 2: 3: 4: 5. Already, the second peak is out of the range. The “true” peak height was calculated by scaling of the Model (concentation = 1) peak height.

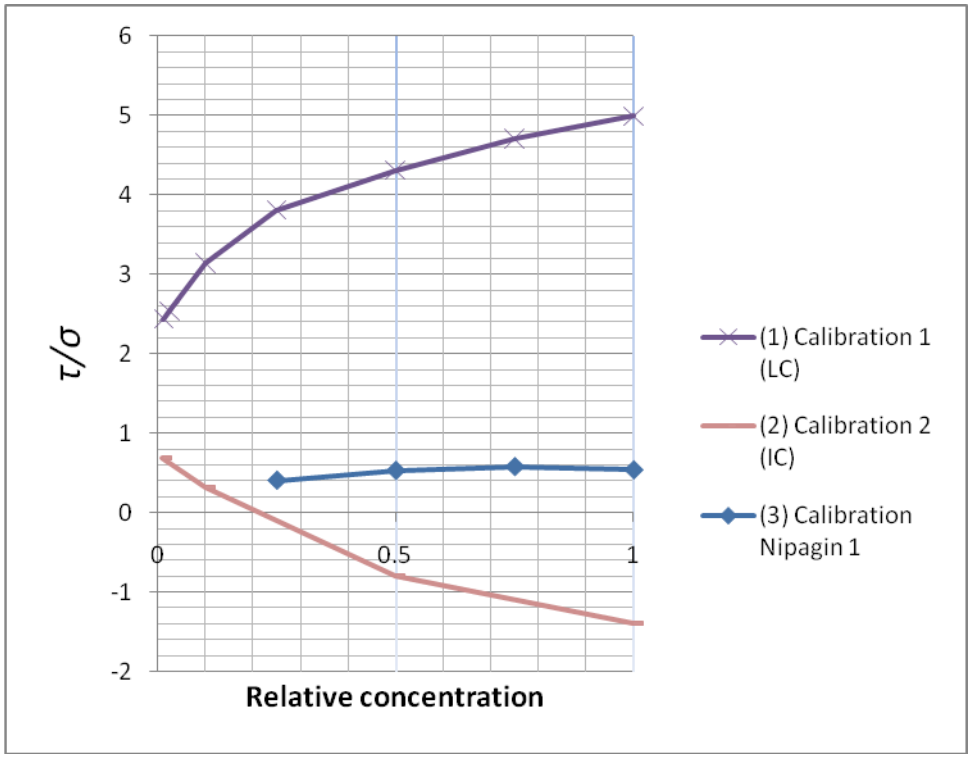

Figure 7. Dependence of peak shape on the concentration. Calibration 1 and 2 are the extreme cases found after investigation of more than a hundred calibrations of different types.

Conclusions:

1. Peaks may be reconstructed with reasonable accuracy for up to 4-times overload.
2. Area is reconstructed better than height.
3. Reconstruction using known peak shape may give good results, but monitoring of peak shape while calibration is required in this case.
4. Column overload cannot be accounted for by this technology due to peak shape distortion.
5. The technology can be used for getting an estimate of required dilution on early steps of method development or in the case of big error in concentration. Numerical results may be suitable for internal use of the investigation laboratory.

References

[1] McWilliam, I. G.; Bolton, H. C. Anal. Chem. 1969, 41, 1755-1762.

[2] Delley, R. *Anal. Chem*. 1985, 57, 388