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Fourier transform assisted deconvolution of skewed peaks in complex multi-dimensional chromatograms

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ABSTRACT

Lower order peak moments of individual peaks in heavily fused peak clusters can be determined by fitting peak models to the experimental data. The success of such an approach depends on two main aspects: the generation of meaningful initial estimates on the number and position of the peaks, and the choice of a suitable peak model. For the detection of meaningful peaks in multi-dimensional chromatograms, a fast data scanning algorithm was combined with prior resolution enhancement through the reduction of column and system broadening effects with the help of two-dimensional fast Fourier transforms. To capture the shape of skewed peaks in multi-dimensional chromatograms a formalism for the accurate calculation of exponentially modified Gaussian peaks, one of the most popular models for skewed peaks, was extended for direct fitting of two-dimensional data. The method is demonstrated to successfully identify and deconvolute peaks hidden in strongly fused peak clusters. Incorporation of automatic analysis and reporting of the statistics of the fitted peak parameters and calculated properties allows to easily identify in which regions of the chromatograms additional resolution is required for robust quantification.
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1. Introduction

Chromatography is one of the most common techniques in analytical laboratories, especially for the analysis of mixtures of larger organic molecules. Its output is typically presented in the form of chromatograms, the intensity of a detector signal over the time of a separation in which each component is represented by a peak. The amplitude of the peak reflects the concentration of the components in relation to the detector sensitivity. The shape of the peak on the other hand is determined by the complex interplay of mass-transfer and adsorption phenomena occurring in the column and in the system dead-volume [1]. In the ideal case, i.e. where the components of interest are fully resolved, the interpretation of these chromatograms is relatively straightforward as lower statistical peak moments, such as the area (0th-moment) and average retention time (1st moment), can be calculated accurately by simple integrators or even graphically [2]. Where peaks are not fully resolved, straightforward approaches such as perpendicular drop or tangent skim, may still lead to reasonable results for symmetrical peaks with limited overlap [3]. In practice peaks may often be skewed due to slow mass transfer or extra-column effects, that can lead to large errors during chromatogram analysis [3].

One of the most wide-spread approaches to solving the problem of overlapping skewed peaks is multivariate curve resolution (MCR) [4]. MCR utilizes the bilinear character of spectroscopic chromatograms [5], i.e. that the recorded chromatogram is a linear combination of the concentration profiles of the present species and their respective absorption properties. MCR has been demonstrated to be both effective for mixtures where the single components absorption spectra are known [6] and unknown [7], though in the latter the statistical uncertainties of the obtained results increase with the number of components present. In contrast to MCR, hard-modeling techniques focus only on the concentration profiles and require only univariate data. The two techniques are highly compatible and can compensate for the short-comings of each other [8].

In this study we introduce a hard-modeling approach for the deconvolution of complex two-dimensional chromatograms. A special focus lies on the generation of good initial estimates with the help of Fourier transforms. The results are subjected to rigorous statistical analysis to identify the regions where the hard-modeling

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approach by itself can lead to sufficiently robust results and where the multivariate techniques might be necessary.

2. Theory

Over the years a huge library of peak models has been developed, many of which can account for peak asymmetries [9]. Probably one of the most popular models for the description of asymmetrical chromatographic peaks is the exponentially modified Gaussian distribution (EMG). Its popularity is, at least, partly based on the relative physical significance of its parameters: the variance can be related to the peak broadening caused by axial dispersion, whereas the exponential decay is a reasonable model to capture dead-volume effects. An additional advantage of the EMG is that it requires a relatively small number of parameters to be able to describe a large variety of peak shapes, from almost perfectly Gaussian to heavily tailing peaks with sharp fronts. The EMG can be expressed in many mathematically equivalent ways that may lead to large errors when calculated numerically for certain parameter ranges. Kalambet et al. [10] introduced a simple decision parameter z to guide in the selection of the form of the EMG to use for accurate numerical calculation. For a single peak this decision factor can be expressed as

$$z = \frac{1}{\sqrt{2}} \cdot \left(\frac{\mu - x}{\sigma} + \frac{\sigma}{\tau} \right) \tag{1}$$

where x is the input variable, for chromatography typically the elution time or volume. The mode of the Gaussian constituent is given as μ, the Gaussian variance σ and the relaxation parameter of the exponential decay as τ. The equation best suited for numerical calculation of the EMG is then for $z < 0$

$$F(x) = h \cdot \sqrt{\frac{\pi}{2}} \cdot \frac{\sigma}{\tau} \cdot \exp\left(\frac{\mu - x}{\tau} + \frac{\sigma^2}{2\tau^2} \right) \cdot \operatorname{erfc}\left(\frac{1}{\sqrt{2}} \cdot \left(\frac{\mu - x}{\sigma} + \frac{\sigma}{\tau} \right) \right) \tag{2}$$

and for $z \geq 0$

$$F(x) = h \cdot \sqrt{\frac{\pi}{2}} \cdot \frac{\sigma}{\tau} \cdot \exp\left(\frac{-(\mu - x)^2}{2\sigma^2} \right) \cdot \operatorname{erfcx}\left(\frac{1}{\sqrt{2}} \cdot \left(\frac{\mu - x}{\sigma} + \frac{\sigma}{\tau} \right) \right) \tag{3}$$

with h being the height of the unmodified Gaussian.

Once such a suitable peak model has been identified, numerical optimizers have been shown to be able to fit them to experimental data [11]. The EMG has been demonstrated to be suitable for optimizer based fitting and deconvolution of most chromatographic peaks providing the observed peak tailing is not too pronounced. In these cases the polynomial modified Gaussian (PMG) shows better fitting capability [12]. Besides the suitability of the peak shape, knowledge of the number of peaks fused in the chromatogram and their relative positons were identified as critical parameters in the success of optimizer based deconvolution [13]. Depending on the complexity of the chromatograms, identifying the number and positions of possible peaks is not a trivial task. To avoid operator to operator variation, especially in quality control environments, it is preferable to have this operation performed by peak detection algorithms. Two popular peak detection approaches are simple local maxima search algorithms, that closely resemble a human looking for visually distinguishable peaks, and analysis of higher-order derivatives of the measurement signal. The latter has been shown to be able to recognize more peaks, especially such hidden in shoulders of larger peaks, but is highly sensitive to noise in the original signal [14,15].

A more robust approach to increase the probability to observe well resolved peaks is to increase the system's peak capacity [16]. This can be achieved by increasing the efficiency of the used columns and reduction of extra-column effects, but most effectively by increasing the number of orthogonal separation dimensions [17]. The principles for the interpretation of these multi-dimensional chromatograms remain the same. For practical reasons, the dimensionality of comprehensive separations is often limited to two orthogonal methods, even when performed in offline mode [18]. As a peak model for two-dimensional chromatography the Kalambet et al. [10] system of equations for the description of EMG shaped peaks can be extended by a second dimension. The general equation to describe a fused set of n two-dimensional EMG distributions (2D-mEMG) is then given by

$$F(x,y) = \sum_{1}^{n} \left\{ h_i \cdot \frac{\pi}{2} \cdot \frac{\sigma_{x,i} \cdot \sigma_{y,i}}{\tau_{x,i} \cdot \tau_{y,i}} \cdot co_{x,i} \cdot co_{y,i} \right\} \tag{4}$$

where $co_{x,i}$ and $co_{y,i}$ are co-factors that change depending on the peak and parameter range. Similar to the one-dimensional case the equation for the accurate calculation of the cofactors can be chosen by decision variables:

$$z_{x,i} = \frac{1}{\sqrt{2}} \cdot \left(\frac{\mu_{x,i} - x}{\sigma_{x,i}} + \frac{\sigma_{x,i}}{\tau_{x,i}} \right) \tag{5}$$

$$z_{y,i} = \frac{1}{\sqrt{2}} \cdot \left(\frac{\mu_{y,i} - y}{\sigma_{y,i}} + \frac{\sigma_{y,i}}{\tau_{y,i}} \right) \tag{6}$$

Similar to the one-dimensional case the co-factors for the first dimension are for $z_{x,i} < 0$

$$co_{x,i} = \exp\left(\frac{\mu_{x,i} - x}{\tau_{x,i}} + \frac{{\sigma_{x,i}}^2}{2{\tau_{x,i}}^2} \right) \cdot \operatorname{erfc}(z_{x,i}) \tag{7}$$

and for $z_{x,i} \geq 0$

$$co_{x,i} = \exp\left(\frac{-(\mu_{x,i} - x)^2}{2{\sigma_{x,i}}^2} \right) \cdot \operatorname{erfcx}(z_{x,i}) \tag{8}$$

Analog to the first dimension, the cofactors for the second dimension are for $z_{y,i} < 0$

$$co_{y,i} = \exp\left(\frac{\mu_{y,i} - y}{\tau_{y,i}} + \frac{{\sigma_{y,i}}^2}{2{\tau_{y,i}}^2} \right) \cdot \operatorname{erfc}(z_{y,i}) \tag{9}$$

and for $z_{y,i} \geq 0$

$$co_{y,i} = \exp\left(\frac{-(\mu_{y,i} - y)^2}{2{\sigma_{y,i}}^2} \right) \cdot \operatorname{erfcx}(z_{y,i}) \tag{10}$$

When viewing the system of Eqs. (4)–(10) it becomes apparent that there is no built in correlation between the first and second dimensions. As a result the peak model should preferably be used to describe systems where the dimensions consist of orthogonal methods. This restriction to the application of the peak model is deemed acceptable, as orthogonality of the separation dimensions is an important part of the design paradigm of multi-dimensional chromatography systems [19]. It should also be noted that fitting multiple peaks to a single chromatogram approach also assumes that the chromatogram is the result of the linear addition of the single component contributions to the final recorded chromatogram, a condition only met when the used detector is strictly operated within its linear response range. When it is no longer feasible to improve the separation system on a technical level, there is the possibility to virtually reduce the contribution of band-broadening and extra column effects. This effect can be achieved with the help of Fourier transformations [20]. Deconvolution by means of the Fourier transforms has been shown to have a suitable sharpening effect on chromatograms with EMG shaped peaks [21]. The characteristics of applying the Fourier transformation in the form of fast Fourier transform (FFT) algorithms to real experimental chromatograms has been studied thoroughly [22]. Due to the introduction of artefacts such as small negative side-lobes, and slight shifts in peak retention patterns, research on the use of Fourier

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