Alignment of chromatograms to facilitate visual comparison of data collected at different points in time or with different instruments

Patrik Petersson[1], Annika Sjölander[1], Melvin R Euerby[2,3], Andrey Vazhentsev[4] and Karim Kassam[4]

[1]Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark

[2]Hichrom Ltd, 1 The Markham Centre, Station Road, Theale, Reading Berkshire, RG7 4PE, England

[3]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow, G40RE

[4]Advanced Chemistry Development (ACD/Labs), 8 King Street East, Suite 107, Toronto, M5C 1B5, Canada.

Introduction

Data collected at different time points or with different instruments, e.g. during the stability studies conducted during the development of pharmaceuticals, will differ slightly due to:

- Column or mobile phase batch to batch variation
- Differences between instruments e.g. column thermostat design, dwell volume …

This will result in small:

- Shifts in retention time
- Changes in selectivity
- Differences in signal intensity
- Differences in the shape of the base-line.

Thereby it becomes difficult to visually spot differences between the chromatograms (Fig.1)

Current chromatographic data software (CDS) does to our best knowledge not offer a solution to the problem.

Figure 1. Chromatograms obtained at 0, 4, 8 and 11 weeks during an accelerated stability study illustrating the problem.

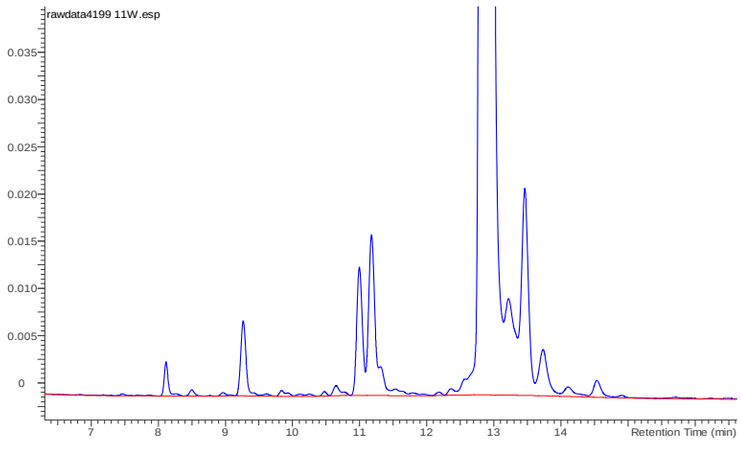

Figure 2. Fitting and subtraction of a base-line (spline function) followed by a naming of peaks to be used for alignment (P1…Pk).

Results and Discussion

The alignment/normalisation procedure outlined below was implemented in an alpha version of ACD/Chrom Workbook 2015 [1].

- Export of chromatograms from CDS as CSV-files
- Import of CSV-chromatograms into software in which the alignment is performed
- Fitting and subtraction of base-line (Spline function, Fig. 2)
- Naming of peaks that should be used for alignment typically a main peak (P1) and a few impurities before (P2…Pi) and after the main peak (Pj…Pk)
- Selection of a reference chromatogram
- Normalisation of the signal axis to obtain the same height of the main peak (P1) in all chromatograms
- A piecewise linear expansion/contraction of the time axis to obtain the same retention for the impurities selected for alignment (P2-Pk, Fig. 3)
- Generation of an overlay to allow a visual comparison and thereby spot impurities that change in intensity (Figs. 4 and 5)

Evaluations have been made with simulated as well as real data sets. Fig. 1,2 and 4 depict data from an accelerated stability study conducted on a proprietary Novo Nordisk A/S peptide. The data was collected after 0, 4, 8 and 11 weeks using the same LC instrument and column but different batches of mobile phase.

In addition to stability studies another application area could be comparisons of chromatograms obtained during translation of methods between different chromatographic formats where the run times differ, *e.g.* from HPLC to UHPLC.

The ACD/Chrom Workbook user interface is depicted in Fig. 5.

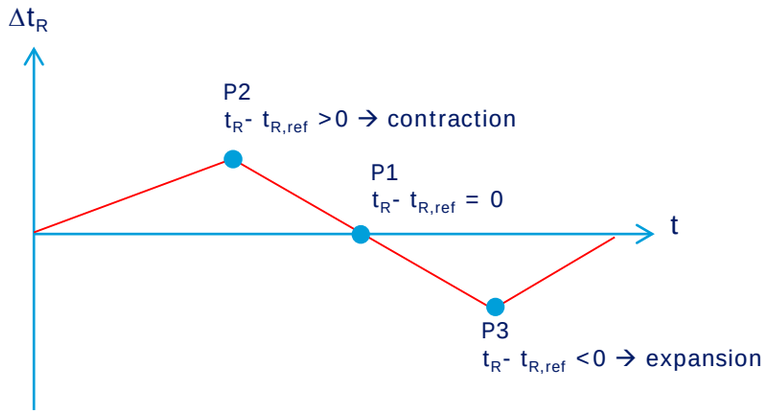

Figure 3. A simplified illustration of the piecewise linear expansion/ contraction of the x-axis to align chromatograms.

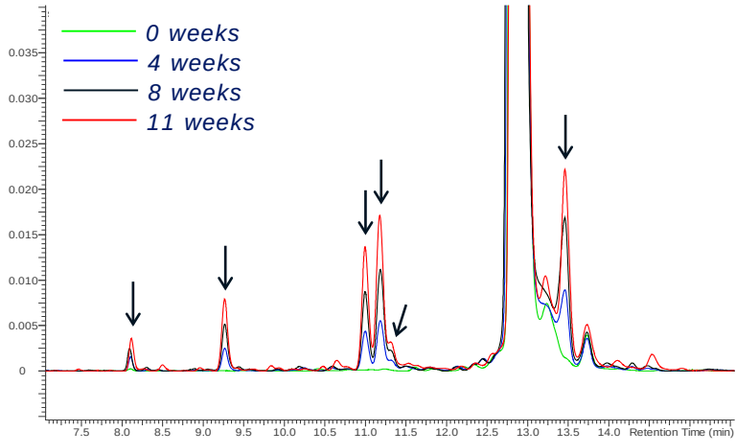

Figure 4. Aligned and normalised chromatograms. Obvious degradation products indicated with arrows.

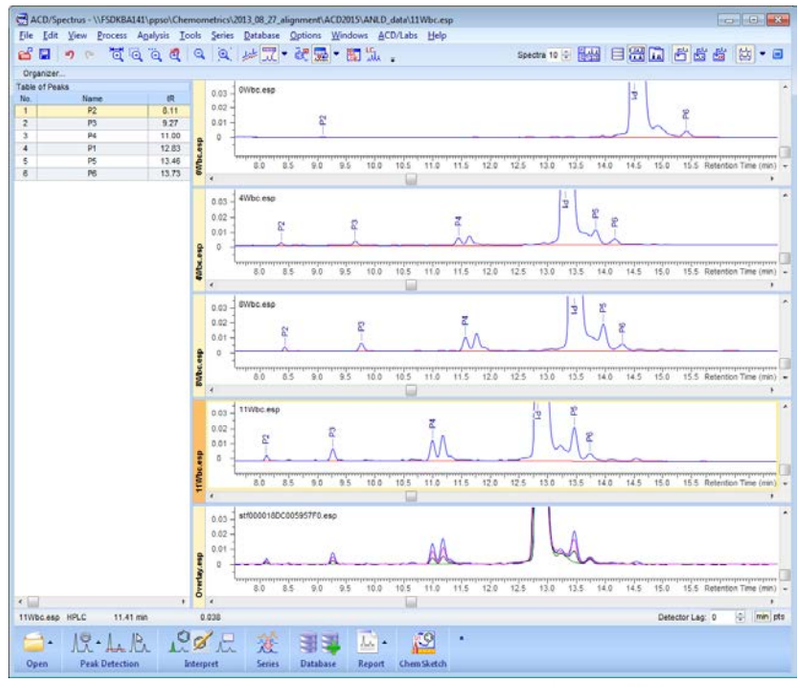

Figure 5. The ACD/Chrom Workbook user interface. All named components P1-P6 have been used in the alignment procedure. The 11 week data was used as a reference chromatogram.

References

[1] ACD/Chrom Workbook 2015 (modified alpha version Feb 2015), Advanced Chemistry Development (ACD/Labs), Toronto, Canada, www.acdlabs.com