UNIVERSITY OF PÉCS
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THE APPLICATION OF TWO-DIMENSIONAL CORRELATION IN CHROMATOGRAPHY AND THE INTRODUCTION TO ALTERATION ANALYSIS

PhD thesis

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1 Introduction

In the last few decades chemometrics had a major role in the progress of analytical chemistry. Besides the advancement of lab equipment and instruments, there is a huge demand for constantly creating more sophisticated methods to analyse the ever increasing amount of data. The evolution of computer science allowed the data, generated in the labs, to rapidly evolve from simple graphs to complex, multi-dimensional sets, which rendered the day-to-day use of evaluating software not just recommended but mandatory.

The expectations for chemometric methods have two – in some way contradictory – cornerstones: be able to analyse the more and more advanced data sets; and create simple, easily understandable results. There is, however, a field in chemometrics which seems to incorporate these goals effortlessly, called two-dimensional correlation analysis (2DCOR). This method was built to extend the possibilities of infrared spectroscopy (IR) with some of the two-dimensional concept used in nuclear magnetic resonance spectroscopy (NMR). It was a great success and later proved to be useful with many probes other than IR. Despite the rapid growth of the field, 2DCOR operated mostly in spectroscopy, only a few attempts have been made outside that, however we strongly believe that its basic concept can be advantageous in chromatography as well.

In the thesis we describe 2DCOR's origins, how it became a versatile, widely used chemometric method and its way from IR through other probes to chromatography. We give details of the mathematical background for both 2DCOR and our new method. Furthermore our studies are presented, where 2DCOR's abilities are being tested on computer generated series of chromatograms; our new method is introduced, in silico experiments detail its properties with 3D datasets and it is compared to 2DCOR in 2D. At last two practical examples show the advantages of methods used in chromatography. The first one presents 2DCOR's performance compared to principal component analysis (PCA) in a reproducibility study of chromatographic columns, whereas the other focuses on concentration changes in supercritical fluid chromatography (SFC) measurements.

2 Aims

The main aims of this work were:

- exploration of the possibilities, attributes and details of 2DCOR in the field of chromatography;
- extension of 2DCOR to three-dimensional datasets where a series of two-dimensional measurements - instead of the original one-dimensional - are evaluated;
- building a new method which uses the properties of 2DCOR, but is able to work with three-dimensional datasets and is fine-tuned to use in chromatography;
- comparison of 2DCOR and our new method on two-dimensional datasets where a series of one-dimensional measurements are used;
- proving the capability of 2DCOR in chromatography by applying it to measured chromatograms and comparing it to other chemometric methods;
- demonstrate the practical use of our new method on measured chromatograms and highlight its advantages in the evaluation of complex chromatographic problems.

3 Experimental and methods

An extensive part of our work was taken up by creating the computational background for the experiments. This thesis does not focus on the programming aspect, however we want to emphasize here that no commercial software was used, every script was written by the author. All calculations were executed in the programming language R [1] with the help of Rstudio software [2].

Chromatographic peaks were generated with the exponentially modified gaussian peak (EMG). The perturbation-induced changes in the series of chromatograms were simulated by changing one parameter of the EMG peaks along the second dimension of the dataset - between the chromatograms - where the perturbation occurs.

For the reproducibility experiments Felinger et al. studied the reproducibility of chromatographic retention data on reversed-phased high performance liquid chromatography (HPLC) columns, and identified the factors that influenced the reproducibility [3]. The systematically measured data were provided by Kele et al. [4, 5].

Continuing the work of Kele et al. [4–9], Gritti et al. studied the reproducibility of HPLC columns under nonlinear conditions, and determined the isotherms of overloaded band profiles [10, 11]. Felinger et al. in turn examined the reproducibility of the equilibrium isotherms with principal component analysis [12].

The thesis presents two experiments with measured data aimed to highlight the advantages of Alteration Analysis (ALA). The first one is constructed by changing the concentration of seven compounds, throughout a series of chromatograms and then comparing the results to computer generated data. The second experiment has the same conditions except the sample concentrations were permanently 0.4 mg/ml and the solvent composition was changed. This perturbation was started at 0:100% methanol:acetonitrile ratio and ended at 100:0% with 10% steps.

4 Results and discussions

Two-dimensional correlation analysis (2DCOR) is a well-established tool in spectroscopy, however, its chromatographic use has not become widespread. The original concept allows that basically any kind of data can be used in the calculations. So in theory the change from spectra to chromatograms has no effect on the outcome. In practice the two types of data naturally have differences and the basic properties of correlation maps from chromatograms have not been explored. The advantages of computer generated data allows that the constants of EMG curves can be modified with precision and every aspect of change with chromatographic peaks can be evaluated. The thesis deals with a great variety of these scenarios and systematically shows the related patterns in the correlation maps.

There are a lot of opportunities in chromatography to expand the original concept of 2DCOR. The most interesting one is to apply correlation maps to 3D datasets made by using a single perturbation on 2D chromatograms. Our solution was to create ALA which provides the essential properties of 2DCOR but simplifies the calculations and visualization, thus higher dimensional data can be utilized. The properties of alteration maps are displayed in the thesis with the same system as 2DCOR maps.

ALA is a great tool to expand the boundaries of 2DCOR, however, this method is capable of much more, because it is able to use original 2D datasets, just like correlation maps, and by the simplification the results are way easier to interpret, because they are linear graphs instead of pseudo-3D contour-plots. This opportunity allows the step by step comparison of the two methods. The same experiments were rigorously applied to 2D ALA as well. The results show that our novel method is capable of withstand the challenges risen by 2DCOR, which means it does not just expand the concept to higher dimensions, but can be a simpler, more efficient alternative.

Besides the in silico experiments measured chromatograms were also put to the test. 2DCOR was used in two reproducibility experiment of HPLC columns. The results were compared to the original experiments where PCA was used.

In the first one, retention properties of a mixture with eight components were studied. We looked at the chromatograms on every column separately and all the chromatograms together. The challenge in this experiment was that there were only minor differences between measurements. Despite the hardship, 2DCOR delivered easily understandable, detailed visual information. The conclusion was that the reproducibility of these columns are astonishing, only small fluctuations of retention times could be seen. PCA made the same remarks, so compared to the reference our method is proven to be equally useful.

The second experiment dealt with overloaded band profiles and isotherm parameters were the main interest. Similar to the latter, 2DCOR could show the same results as PCA. Besides, our method provided a unique approach where a constructed data was made combining the seven individual chromatograms. This way seven separate evaluations could be swapped for just one, shortening the processing time.

ALA was also further tested on two experiments with measured chromatograms. At first a series of test mixtures was prepared where the components concentrations were separately changed in similar manner as the previous in silico experiments. The solutions were measured and replicated with computer generated data. The two set of alteration maps were compared and it was demonstrated that theoretical workings of ALA can be planted into practice, because between the two data only the minor fluctuations of retention times meant some difference.

Last but not least ALA was applied to a series of measurements where the ratio of methanol in the solvent was increased. The maps showed –with only one graph– how the peak of methanol increases and migrates through the chromatogram and how its concentration affects the detection of other components.

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5 Thesis points

1. We introduced a novel method called Alteration Analysis (ALA), in order to extend two-dimensional correlation analysis (2DCOR) to three-dimensional datasets where a series of two-dimensional measurements – instead of the original one-dimensional – are evaluated.

2. Our method, ALA was built to use the properties of 2DCOR, but it made able to work with three-dimensional datasets and fine-tuned to use in chromatography, because of its focus on individual changes rather than the connection between them. These benefits were explored by a series of examples which showed that ALA can work equally on one and two-dimensional measurements, but more importantly that it made possible for the field of 2DCOR the analysis of higher dimensional datasets.

3. We made an intensive comparison of our method and 2DCOR on two-dimensional datasets where a series of one-dimensional measurements are used. We proved that ALA is more than capable of competing with its predecessor, because it has many advantages to overcome its one disadvantage. It can work with 3D datasets, it can show the absolute direction of changes instead of only relative ones and it can be understood much easier with its simpler graphs.

4. We tested the capability of 2DCOR in chromatography by applying it to measured chromatograms and comparing it to other chemometric methods. We clarified that it can be applied well in this field and it delivered the same informations about the reproducibility of HPLC columns as PCA.

5. We demonstrated the practical use of our new method, ALA on measured chromatograms and its advantages were highlighted in the evaluation of complex chromatographic problems. The information in ALA maps of measured chromatograms were compared to in silico experiments and only negligible differences arose, meaning the theory behind ALA can be put into good use in practical experiments. Furthermore, the effect of methanol was shown on ALA maps in SFC measurements.

Publications

Publications related to this thesis

1. **Simon, J.**, Felinger, A., Two-dimensional correlation analysis of the reproducibility of high-performance liquid chromatography columns, J. Chromatogr. A, 2015, 1384, 115–123

2. **Simon, J.**, Felinger, A., Correlation analysis on 3D data – Introducing the alteration analysis, Chemometr. Intell. Lab. Syst., 2016, 158, 54–60

3. **Simon, J.**, Felinger, A., Exploring the changes in a series of measurements – The comparison of the two-dimensional correlation analysis and the alteration analysis, Chemometr. Intell. Lab. Syst., 2017, 168, 28–37

Posters and presentations related to this thesis

1. **Simon, J.**, Felinger, A., Two-dimensional correlation in chromatography, 9th Balaton Symposium on High-Performance Separation Methods, 2013. 09.04–09.06., Siófok

2. **Simon, J.**, Felinger, A., Kétdimenziós korreláció alkalmazása a kromatográfiában, XXXVI. Kémiai Előadói Napok, 2013. 10.28–10.30., Szeged

3. **Simon, J.**, Felinger, A., Two-dimensional correlation in chromatography, 10th János Szentágothai Transdisciplinary Conference and Student Competition, 2013. 11.04–11.05., Pécs

4. **Simon, J.**, Felinger, A., Correlation on 3D data – Alteration Analysis, The 8th International Symposium on Two-Dimensional Correlation Spectroscopy, 2015. 07.08–07.11., Vienna, Austria

5. **Simon, J.**, Felinger, A., Correlation on 3D data – Alteration Analysis, 10th Balaton Symposium on High-Performance Separation Methods, 2015. 09.02–09.04., Siófok

6. **Simon, J.**, Felinger, A., Correlation on 3D data – Alteration Analysis, Conferentia Chemometrica, 2015. 09.13–09.16., Budapest

7. **Simon, J.**, Felinger, A., Alternation Analysis: An alternative to 2DCOR, The 9th International Symposium on Two-Dimensional Correlation Spectroscopy, 2017. 06.07–06.10., Victoria, Canada

8. **Simon, J.**, Felinger, A., An alternative to two-dimensional correlation analysis: Alteration Analysis, Conferentia Chemometrica, 2017. 09.03–09.06., Gyöngyös, Farkasmály

9. **Simon, J.**, Felinger, A., Correlation Analysis on a Series of 1D and 2D Chromatograms, 11th Balaton Symposium on High-Performance Separation Methods, 2017. 09.06–09.08., Siófok

Posters and presentations not related to this thesis

1. **Simon, J.**, Felinger, A., Modelling the 2D correlation in chromatography with exponentially modified Gaussian peak shape, III. Interdisciplinary Conference, 2014. 04.15–04.17., Pécs

2. **Simon, J.**, Felinger, A., Modelling the 2D correlation in chromatography with exponentially modified Gaussian peak shape, 30th International Symposium on Microscale Bioseparations, 2014. 04.27–05.01., Pécs

3. **Simon, J.**, Felinger, A., Enhancement of chromatographic peak determinations by two-dimensional correlation, 30th International Symposium on Chromatography, 2014. 09.14–09.18., Salzburg, Austria

4. **Simon, J.**, Felinger, A., A kromatográfiás kétdimenziós korrelációs térképek leírása módosított Gauss-görbékkel, Elválasztástudományi Vándorgyűlés, 2014. 11.12–11.14., Egerszalók

5. **Simon, J.**, Lambert, N.,Felinger, A., Kromatográfiás hatékonyság vizsgálata alterációs analízissel, Elválasztástudományi Vándorgyűlés, 2016. 11.09–11.11., Kecskemét