Comparative study of different third-order data generation approaches / HPLC-FD data analysis

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INTRODUCTION

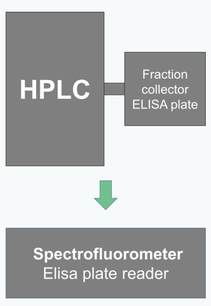
Over the last years, it has been demonstrated that the increase of multi-way data dimensions has a positive impact on analytical figures of merit, e.g. higher sensitivity, lower limits of detection and quantitation, better selectivity, among others. First- and second-order data analyses have become excellent tools for the resolution of complex samples which would result experimentally challenging from the univariate calibration standpoint. On the other hand, even though no additional analytical advantages have been yet proved, third-order data analysis for analytical applications constitutes a field worth to be explored. Although multidimensional instrumental signals are easy to be obtained with the available modern instrumentation, and several chemometric algorithms have been successfully developed to solve multi-way data problems, the way in which the multi-way data are generated may have a significant effect on the final results. In this work, a comparative study of different third-order data generation approaches was carried out. Three methods based on identical liquid chromatographic conditions but coupled to different emission and excitation fluorescence detection system were developed for the analysis of antibiotics in aqueous matrices.

EXPERIMENTAL PROCEDURES

All experiments were performed under same chromatographic conditions, by using an Agilent 1100 LC instrument in isocratic mode.

- Analytical column Zorbax XDB-C18, 75×4.6mm, 3.5µm.
- Mobile phase: HAc buffer pH4.0:ACN:MeOH mixture (71:9:20).
- Column temperature at 35 ºC.

1) Collection of fractions



Flow rate: 2 mL min^{-1}

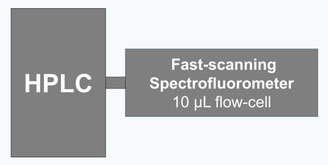
- For each sample:
  - 1 Chromatographic run collecting 25 fractions, every 2 s = 2 min
  - 25 EEMs = 40 min.

2) Multi-chromatographic run / Multi-excitation

Flow rate: 2 mL min^{-1}

- For each sample:
  - 10 Chromatographic runs recording time-emission matrix at fixed excitation wavelength (different excitation wavelength for each run) = 40 min

3) On-line EEM detection

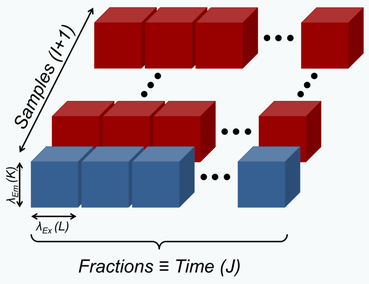


Flow rate: 0.5 mL min^{-1}

- For each sample:
  - 1 Chromatographic run recording 25 sequential EEMs = 7 min

DATA ANALYSIS

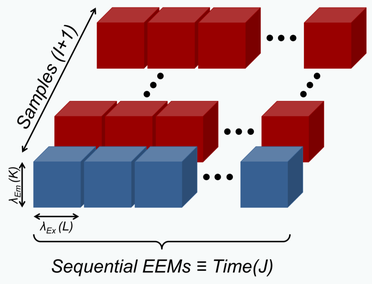
Analysis time per sample	40 min	(HPLC + FD)= 45 min
Matrix size	(*J*×*K*×*L*): 10×121×25	(*J*×*K*×*L*): 25×17×25
Data pre-processing:	TEM* smoothing Peak alignment TEM baseline correction	EEM* smoothing
Data processing:	PARAFAC, *A*PARAFAC, MCR-ALS, U-PLS/RTL	PARAFAC, *A*PARAFAC, MCR-ALS, U-PLS/RTL



Chromatographic run ≡ λ_{Ex} (J)

*TEM= Time-Emission Matrix

Analysis time per sample	(HPLC + FD)= 7 min
Matrix size	(*J*×*K*×*L*): 15×28×15
Data pre-processing:	TEM smoothing
Data processing:	????



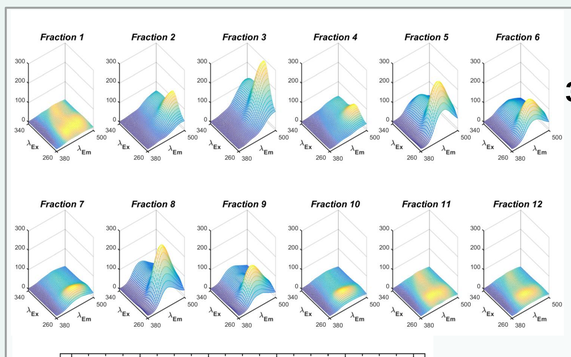
Sequential EEMs ≡ Time(J)

*EEM= Excitation-Emission Matrix

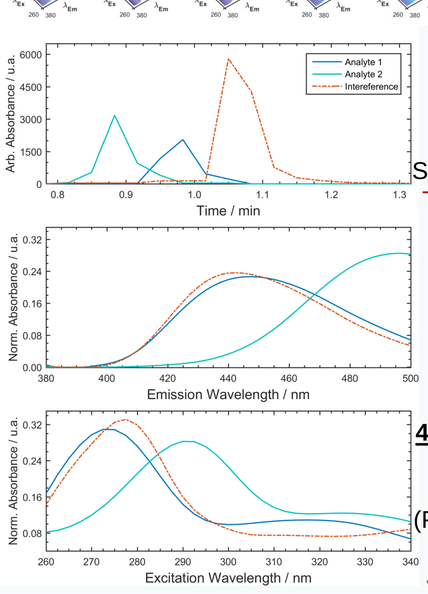
RESULTS and DISCUSSION

In order to demonstrate the complexity of the system, the data pre-processing just included smoothing. No peak alignment or baseline correction was performed. The figures show PARAFAC results.

3 analytes
3 components



1 components for each analyte



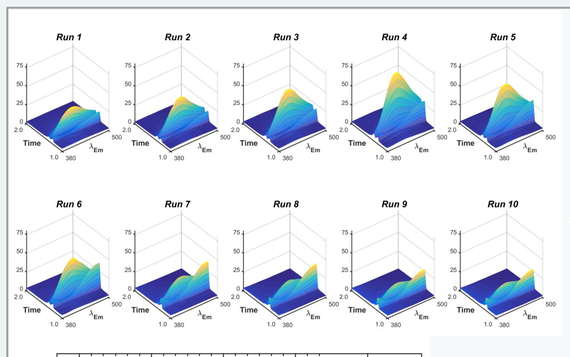
General features
Minimal data pre-processing
Simple data processing
Semi-automated data generation
Three instruments are required
Time consuming

3rd order data (TEEC*)
Trilinear data
High selectivity in the three modes

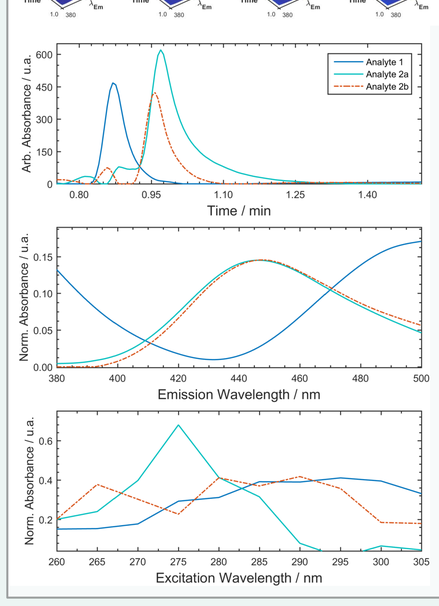
4-way data array (Conc-TEEC)
Non-quadrilinear
Breaking mode=Elution time
(Peak shifting between samples)

*TEEC= Time-Excitation-Emission Cube

2 analytes
3 components



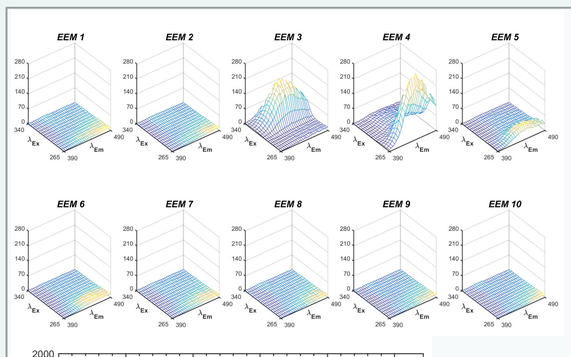
1 component for 1 analyte (blue)
2 components for 1 analyte (cyan and red) due to peak shift within sample



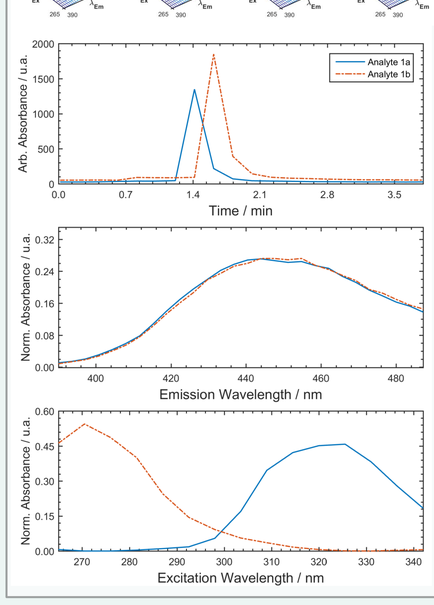
General features
Complex data pre-processing
Simple data processing
Automated data generation
One instrument is required
Time consuming

3rd order data (TEEC*)
Non-trilinear data
Breaking mode=Elution time
(Peak shifting within sample, leading to a trilinear break in excitation mode)
High selectivity in two modes
Low selectivity in one mode

1 analyte
2 components



2 components for 1 analyte (blue and red) due to the strong dependency of excitation recording with concentration.



General features
Minimal data pre-processing
Very complex data processing
Automated data generation
Two instruments are required
Non-time consuming

3rd order data (TEEC*)
Non-trilinear data
Breaking mode=Excitation
(Excitation is strongly affected by concentration/elution time)
High selectivity in the three modes

CONCLUSIONS

The three techniques allow to obtain third-order data in a simple way without using sophisticated instrumentation. ***Method 1*** becomes the selected one to generate this kind of data despite of the instrumentation requirement and the time consumption. The obtained data is simple and well-known algorithm are used for its modelling. *This methodology could be improved by using a fast-scanning spectrofluorometer.* On the other hand, just a HPLC with fast-scanning fluorescence detector is required for ***Method 2***. However, due to its high time consumption, it is not appropriate for long chromatographic runs and analysis of unstable compounds. Furthermore, complex data pre-processing could be demanded prior to data modelling. Last but not least, the **Method 3** shows the most promising methodology for 3rd-order data generation, considering its equipment simplicity and fast data acquisition. Nevertheless, *it is strongly necessary to develop an algorithm that allows to model this data, taking into account its particular characteristics.*

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