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Short communication

MCR-ALS as resolution method for coeluting analytes in a challenging LC-DAD analytical procedure

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Introduction

Forced degradation studies, as a crucial part of the pharmaceutical development, provide information for the degradation pathways of the active compound and estimate the stability indicating power of the analytical procedures. However, under stress conditions many degradation products chemically very similar to the active compound can be formed, making their chromatographic separation extremely challenging. Often, mass balance cannot be fulfilled since some of the generated impurities cannot be detected due to coelution with the main component or loss of chromophores during the degradation process (Alvarenga and Carneiro 2019).

In this work, pharmaceutical tablet formulation which proved to be quite stable during stability testing, was subjected to extreme temperature conditions (105°C) for a period of 3 days in order to force its degradation. Since a decrease in the main component's assay of around 5% was achieved, but the generated impurities did not account for the total assay loss, the chemometric method Multivariate curve resolution – alternating least squares (MCR-ALS) was applied in order to test its ability to separate potentially coeluted impurities from the API. This method was selected since it simultaneously decomposes the absorbance data matrix into concentration profiles and pure spectra without requiring previous knowledge of impurity identity.

Materials and methods

The analysis was conducted on Dionex Ultimate 3000 UHPLC with Diode Array Detector (Thermo Fisher Scientific, Inc.). The separation was performed on Zorbax Eclipse XDB Phenyl, 150x3mm, 5µm, HPLC column (Agilent Technologies, Inc.), using water and ethanol (HPLC grade, Thermo Fisher Scientific, Inc.), as mobile phase in two step gradient elution program: from 70:30 to 60:40 (*v/v*%) for 10 minutes and then to 20:80 (*v/v*%) for 10 minutes. Flow rate of 0.7 mL/min, column temperature of 40°C and injection volume of 10 µL were used. The UV-spectra were recorded from 190-400 nm with 1 nm resolution. The sample was prepared in concentration of 1 mg/mL, calibration standards in concentration range from 0.002 mg/mL to 0.1 mg/mL and one subtraction standard in concentration of 0.5 mg/mL, by dissolving them in diluent consisted of water and ethanol in ratio 50:50 (*v/v*%). The chromatograms were recorded and extracted from Chromeleon 7 Chromatography Data System sof tware, ver. 7.3.2 (Thermo Fisher Scientific, Inc.).

Chemometric modeling was performed using software Python ver. 3.10.12 with the libraries NumPy ver. 2.2.6 and Matplotlib ver. 3.10.8.

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Results and discussion

Initially MCR-ALS was performed on the raw data, truncated to cover only the elution time window of the main component, but several challenges necessitated sample preprocessing. The significant UV-absorption of the organic eluent, especially at lower wavelengths, together with the gradient elution process which generates a time-varying baseline, produced a poorly shaped concentration initialization matrix for MCR-ALS. In order to remove the baseline interference, the wavelengths lower than 218 nm were removed and the absorbance matrix obtained for pure diluent was subtracted from each standard and the sample. Additionally, the 0.5 mg/mL standard was subtracted from the sample in order to overcome detector saturation by reducing the dominant API signal and preventing it to compromise the sensitivity of the MCR-ALS decomposition for minor components. Before the subtractions, the chromatograms were aligned for any retention time shifts by correlation optimized warping. The pre-processed matrices were stacked vertically in the retention time direction to form an augmented matrix. Singular value decomposition (SVD) of the augmented matrix determined presence of two coeluted components (the API and one impurity). Evolving factor analysis (EFA) was used as initialization strategy, but when performed on the original data, the residual baseline not completely removed with the subtraction process interfered with the determination of components' exact elution time windows. So, in order to completely suppress the slowly varying gradient background, Savitzky-Golay second derivatives (window 11, polynomial order 4) were calculated for the standard block and the sample separately and used for building the concentration initialization matrix with EFA. Then MCR-ALS was performed on the original (non-derivative) augmented matrix. During the iterations the constraints for non-negativity on both concentrations and spectra, unimodality and species correspondence were applied (de Juan et al. 2014). Convergence was achieved with an excellent lack of fit of 0.6515%, total explained variance of 0.999958 and mean residual of 0.011.

The resolved co-eluting impurity spectrum differed from the API spectrum (purity angle of 38.6°), suggesting they are chemically distinct components. A linear calibration curve was constructed from 0.002 to 0.1 mg/mL ($R^2 = 0.999998$) and the impurity concentration was calculated, supposing a relative response factor of 1.

The obtained amount of 1.9% coeluted impurity resolved with MCR-ALS almost doubled the total amount of impurities, since a sum of 2.2% chromatographically separated impurities from API was determined with the method for related and degradation products. When the final total amount of impurities of 4.1% was added to the amount of API calculated with an independent validated assay method, a mass balance of 98.5% was achieved.

Conclusion

These preliminary results indicate that MCR-ALS is a powerful chemometric technique which can be successfully used for separation of coeluted components despite their significant concentration differences and the complexity of the matrix where solvent interference is also present. The algorithm, coupled with several preprocessing techniques, was tested on one sample at one selected degradation condition and the results are promising, but more work is required in order to obtain deeper insights into the degradation pathways and possibly the reaction kinetics. Also, challenging the model with different samples which will introduce additional between-sample variability into the system would be significant for better assessment of its performance on more complex data sets.

References

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