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Short communication

Screening the capability of ATR-FTIR for simultaneous quantification of Vitamin B1, B6 and B12 in powder blend

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Introduction

Fourier Transform Infrared Spectroscopy (FTIR) is a well-established analytical technique, easy-to-use for rapid and non-destructive analysis. Owing to the lack of need for hazardous solvents or reagents in sample preparation, FTIR belongs to the group of environmentally friendly techniques, known as "green analytical techniques" (Fahelelbom et al., 2020). Additionally, the quality of results that this technique produces are comparable to the most powerful techniques, while maintaining extremely low costs for routine analysis and instrument maintenance. The Attenuated total reflectance (ATR) module allows the recording of FTIR spectra of solid and liquid samples directly without any further preparations (Bunaciu et al., 2010).

Coupling FTIR-ATR with smart chemometric tools (Khoshmanesh et al., 2012) such as partial least square regression modeling provides optimum performance for quantitative analysis, besides the rich information provided about the structure of the functional groups of the sample tested. In the case of FTIR-based quantitative analysis, chemometric models are usually built upon predictor variables that are either wavelength-dependent intensities or the linear combination of wavelength-dependent spectral intensities and as such are of immense importance as process analytical tools (PAT) for monitoring content uniformity in solid dosage form production.

The aim of this research was to screen the capability of FTIR-ATR as a PAT for quantification of Vitamins B1, B6 and B12 in a powder blend, using a partial least square (PLS) regression model.

Materials and methods

Materials

The materials used in this study include pure active substances of Vitamin B1 (Thiamine hydrochloride), Vitamin B6 (Pyridoxine Hydrochloride) and Vitamin B12 (Cyanocobalamin). Target concentrations (w/w) for active substances were: 33.311 % for Vitamin B1, 66.622 % for Vitamin B6 and 0.067 % for Vitamin B12.

Equipment and method

For spectrophotometric analysis, a spectrophotometer Bruker FTIR Alpha with an ATR Platinum diamond module was used. The background spectrum within the instrument was recorded prior to the start of each measurement. The powder samples of APIs and matrix formulation samples were measured by placing an approximate quantity of each sample on the surface of the instrument's crystal using a spatula. The ATR pressure arm was lowered to provide good contact between the crystal and sample molecules. In-between each sample application, the ATR crystal was cleaned with a 70% (w/v) aqueous solution of ethanol. Each background and sample measurement was a result of 16 scans in the mid-infrared region (4000–400 cm^{-1}) with a resolution of 4 cm^{-1}. All samples were compressed directly on the ATR crystal with the aid of a standard anvil accessory and all collected data were recorded in absorbance mode, based on the additive nature of Beer's Law. Atmospheric compensation command was applied to eliminate

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disturbing H_2O and/or CO_2 bands in ratio spectra due to different H_2O and CO_2 vapour concentrations in the beam path.

Spectral data were processed using control software Opus 7.5 build, while interpretation of all collected data was carried out by chemometric analysis software SIMCA. The concentration of the Vitamins was employed as a dependent variable (Y), while the corresponding FTIR spectra, as independent (X) variables. All generated spectra were pre-processed with the Standard normal variate (SNV) technique to improve the predictive ability of the chemometric model since optical layer thickness sustainably varies between these types of measurements.

Results and discussion

A series of 22 powder mixtures with varying concentrations from 80 to 120 % of target content for Vitamins B1, B6 and B12 were prepared and analyzed in triplicate (a total of 66 sample scans).

Five main components were employed for building the initial PLS quantification model (R2X=0.78, R2Y=0.86, Q2=0.57). The root mean square error of estimation (RMSEE) were 1.037 % for Vitamin B1, B6 and 0.014% for Vitamin B12. Considering the low target content of Vit. B12, the obtained RMSEE of the quantification model is inappropriate and probably occurs due to the low contact area of the ATR crystal with the analyzed sample which drastically reduces the possibility of scanning a representative sample especially when the analyte is present in such low concentrations. Therefore, it was decided to exclude the content of Vitamin B12 to eliminate its confounding effects in the quantification model. The improved model demonstrated significantly higher R2Y and Q2 (0.96 and 0.82) with RMSEE of 0.56 and 0.55% for Vit B1 and B6, respectively. The VIP plot depicts the main spectral features related to the quantification algorithms for Vit B1 and B6, which aligns with their most prominent spectral bands identified from the spectra of pure substances.

Conclusion

The initial screening experiments revealed the possibilities and drawbacks of the ATR-FTIR in conjunction with PLS modeling as a quantification tool for Vit B1, B6 and B12 in complex powder blends. The low contact area of the ATR crystal clearly poses a limitation for quantitative analysis of low-content compounds (as Vit B12), thus restricting the further development of the model towards the Vit B1 and B6 which produced favorable statistical indicators (high R2Y and low RMSEE). To establish the ATR-FTIR as PAT for powder blend homogeneity monitoring, further experiments using excipients in the powder blends (real production scenario) and a referent analytical technique for quantitative analysis, should be performed.

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