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Short communication

Green gradient RP-HPLC method development based on transformation model

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Introduction

In the recent years, sustainability has become an essential priority in the pharmaceutical industry towards reducing waste generation, improving workplace safety and protecting the environment. The environmental impact of the pharmaceutical analysis is increasingly emphasized by the regulatory frameworks governing solvent use. However, current analytical methods still predominantly rely on conventional toxic solvents, such as methanol (MeOH) and acetonitrile (ACN), leading to growing pressure on the pharmaceutical industry to shift toward more sustainable practices. One of the solutions towards implementing sustainability in the pharmaceutical analysis, is implementing the Green Analytical Chemistry (GAC) principles by substitution of these solvents with a green organic solvent, such as ethanol (EtOH). EtOH has been identified as one of the most widely used green organic solvents in reverse-phase high-performance liquid chromatography (RP-HPLC). Adopting green RP-HPLC methods in the pharmaceutical industry is often hindered by lengthy, waste-intensive redevelopment workflows. Implementing a streamlined transformation model eases this transition, offering a rapid and eco-friendly alternative to the traditional method development process. Existing literature offers a transformation model that provides initial guidance for directly replacing the volumetric fraction (φ, %) of MeOH and of ACN in conventional RP-HPLC methods with the appropriate φ (%) of EtOH, requiring no further changes to the chromatographic conditions compared to the conventional RP-HPLC method. The model is based on the elution strength of EtOH in relation to MeOH and to ACN, intended for isocratic methods on octadecyl silica (C_{18}) columns (Trifunovska et al., 2025).

The aim of this research was to verify whether the established transformation model can be applied for transformation of gradient conventional to green RP-HPLC methods. To test this, a conventional Ph. Eur. method (07/2025:3137) for analyzing dapagliflozin propylene glycol monohydrate (DPM) related substances was selected.

Materials and methods

Selected API and reagents

A reference standard of DPM CRS (Ph. Eur., Y0002487) and dapagliflozin impurity A CRS (Imp. A, Ph. Eur., Y0002486) were used. ACN, trifluoroacetic acid (TFA) and EtOH, all LC grade (Supelco) were used for the preparation of the mobile

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phases and diluents. Regenerated cellulose (RC) membrane filter (Sartorius) 0.45 µm was used for filtration of the solutions.

Chromatographic conditions

The chromatographic analysis was performed on Dionex UltiMate 3000 Method Scouting UHPLC (ThermoFisher Scientific, USA) system, equipped with DAD detector, controlled by the Chromeleon Chromatography Data System (version 7.3.2). Chromatographic conditions were set according to the Ph. Eur. monograph. SunFire C_{18} column (150 mm × 4.6 mm, 3.5 µm) was used, column temperature was set to 30ºC, flow rate of 1.0 mL/min, injection volume of 10µL and detection at 220 nm. The conventional gradient method consisted of mobile phase A (TFA: water for chromatography = 1:2000 *V/V*) and mobile phase B (TFA : ACN = 1:2000 *V/V*). The gradient ramp of the used φ (%) ACN was from 15 – 90%. In the transformed green method, the identical chromatographic conditions were used with a change of ACN in mobile phase B with EtOH, according to the transformation model (Trifunovska et al., 2025).

Preparation of solutions

Analyzed solutions were prepared according to the relevant monograph, by dissolving in ACN, whereas for the green method ACN was replaced with EtOH. The test solution of the API was prepared in concentration of 0.2 mg/mL. The resolution solution contained DPM and Imp. A in concentration of 0.2 mg/mL and 0.16 µg/mL, respectfully.

Results and discussion

The proposed transformation model (Trifunovska et al., 2025) offers two options for substitution of toxic organic eluents: first according to the exact φ (%) of the used organic eluent and second according to the calculated ratios, where φ ACN = 1.03 φ EtOH. According to the first option, φ (%) ACN in mobile phase B was changed with a gradient ramp of EtOH from 20 – 72%, while with the second option with a ramp of 14 – 86%. The system suitability (SS) parameters: retention time (tR), symmetry factor (As), theoretical plates number (N), relative retention time (RRt) and resolution (Rs), were monitored and compared between the conventional and green methods. The obtained values for the SS parameters were comparable between the methods. However, the SS parameters of the green method matched the conventional method much more closely when the transformation was based on the calculated ratios. This aligns with the findings in the literature for the comparison of the elution strength between these solvents (Trifunovska et al., 2025). The t_R of DMP and Imp. A (~23 min and 23.5 min, respectively) obtained with the green method transformed according to the calculated ratios were longer compared to the conventional method (17 min and 17.5 min, respectively). However, the RRt of Imp. A (1.03) for the green method matches the RRt (1.02) obtained with the conventional method. Although the green method yielded lower N, the values were still acceptable, shifting from around 230,000 for DPM and Imp. A in the conventional method to 182,000 in the green method. Comparable Rs between DPM and Imp. A was achieved with both methods (3.0 for conventional vs. 2.8 for green), with similar values that exceeded the monograph`s requirement of 2.5.

Conclusion

This research demonstrates that the proposed model has a potential to be applied for transformation of conventional gradient to green EtOH-based RP-HPLC methods. The gradient method employed a detection wavelength of 220 nm, supporting the suitability of EtOH as an eluent at lower detection wavelengths. The simplicity and practicality of the proposed transformation model, which requires only the selection of the appropriate φ (%) of EtOH from a tabulated dataset, facilitate the implementation of GAC principles within the dynamic environment of the pharmaceutical industry.

References

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