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Short communication

Biopartitioning micellar chromatographic assessment of gastrointestinal absorption of novel hybrid molecules

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Introduction

The pharmacokinetic characterization of structurally complex drug-like molecules is an important component of contemporary medicinal chemistry, particularly for compounds intended for oral administration. Beyond biological activity, further development is strongly influenced by physicochemical properties, membrane-partitioning behavior and the capacity to achieve adequate gastrointestinal absorption (Lipinski et al., 2001).

Biomimetic chromatographic techniques provide experimentally accessible descriptors relevant to the interpretation of absorption-related properties. Among them, biopartitioning micellar chromatography (BMC) has been introduced as a useful model for evaluating analytes distribution between aqueous and biomimetic micellar environments. The method employs reversed-phase liquid chromatography in the presence of micelles, which act as a dynamic pseudo-phase capable of hydrophobic, polar and hydrogen-bonding interactions with analytes (Dobričić et al., 2017).

Coumarin–triazole–isatin hybrids represent a newly synthesized group of structurally diverse heterocyclic molecules with potential biological relevance. Their architecture integrates a coumarin scaffold, a 1,2,3-triazole linker and an isatin fragment, while additional structural diversity is introduced through variation of the coumarin–*O*–alkylene–triazole linker lenght and substitution of the isatin moiety with halogen atoms, methyl or nitro groups (Dimkovski et al., 2025). These modifications may substantially affect lipophilicity, polarity and interaction with biomimetic phases.

The aim of this study was to apply BMC as a complementary chromatographic tool for gastrointestinal (GIT) absorption-related profiling of these hybrids and to relate chromatographic retention to structural variation and micelle/water partitioning behavior.

Materials and methods

BMC was performed on an Agilent 1200 Series HPLC system equipped with a binary pump, manual injector with a 20 μL injection loop, and diode-array detector. Chromatographic analysis was carried out on a Zorbax Extend-C18 column (150 × 4.6 mm, 5 μm) under isocratic elution conditions. The mobile phase consisted of acetonitrile and 0.04 mol/L Brij 35 prepared in 7 mmol/L phosphate buffer pH 5.5, in a ratio of 20:80 (v/v). The column temperature was maintained at 36.5 °C, the flow rate was 1.0 mL/min, and detection was performed at 262 nm.

The investigated compounds and donepezil, used as a reference compound, were initially dissolved in DMSO at a concentration of 2 mg/mL. Prior to

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analysis, the solutions were diluted with the mobile phase to a final concentration of 20 μg/mL, with a final DMSO content of 1% (v/v). Retention times were recorded under the described chromatographic conditions. The retention factor was calculated as $k = (t_R - t_0)/t_0$, where t_R is the retention time of the analyte and t_0 is the column dead time, determined from the DMSO signal. The obtained k values were used as chromatographic descriptors of micelle/water partitioning behavior.

Results and discussion

BMC enabled differentiation of the coumarin–triazole–isatin hybrids according to their interaction with the Brij 35 micellar pseudo-phase. All investigated hybrids showed higher retention than reference compound donepezil. The retention factors of the hybrid molecules ranged from 2.11 ± 0.002 to 9.26 ± 0.172, whereas donepezil showed a markedly lower value of k = 0.64 ± 0.014. This indicates that the synthesized hybrids possess a greater tendency for micelle/water partitioning under the applied chromatographic conditions.

A clear structural influence on retention was observed. Extension of the coumarin–*O*–alkylene–triazole linker generally resulted in increased retention, suggesting that molecular elongation and increased hydrophobic surface enhance interaction with the micellar environment. The shortest *O*-alkylene series (ethylene) showed k values from 2.11 to 6.26, while the longest series (butylene) from 3.82 to 9.26. This trend confirms that spacer length is an important determinant of biomimetic partitioning behavior.

The substitution pattern on the isatin moiety further modulated retention. Halogenated derivatives, particularly bromo- and iodo-substituted analogues, showed the highest k values within the corresponding series, consistent with increased hydrophobicity and polarizability. In contrast, nitro-substituted derivatives displayed lower retention than the heavier halogenated analogues. This may be related to the more polar and strongly electron-withdrawing character of the nitro group, which can reduce the relative contribution of hydrophobic interactions with the micellar phase. Methyl-substituted derivatives generally showed intermediate retention, reflecting a moderate hydrophobic contribution.

From the perspective of GIT-related profiling, BMC retention should be interpreted as a descriptor of partitioning behavior rather than a direct measure of permeability. Moderate to high retention indicates affinity towards biomimetic micellar environments, which may support membrane interaction; however, excessive retention may also reflect pronounced hydrophobicity. Therefore, the obtained k values provide useful comparative information for assessing the balance between aqueous compatibility and membrane affinity within this hybrid series.

Conclusion

Biopartitioning micellar chromatography was applied as a biomimetic approach for GIT absorption-related profiling of coumarin–triazole–isatin hybrid molecules. The obtained retention factors indicated pronounced interaction of the hybrids with the Brij 35 micellar pseudo-phase, influenced by the length of the coumarin–*O*–$(CH_2)_n$–triazole spacer and the nature of the isatin substituent. Overall, BMC provided useful chromatographic descriptors for comparative ADME-oriented assessment of this structurally related compound series.

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