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Short communication

Effect of stationary phase selection on the chromatographic behavior of formoterol fumarate and fluticasone propionate

Gabriela Petrovska-Dimitrievska*, Sanja Serafimova, Biljana Atanasovska, Liljana Ugrinova, Hrisanta Godzo, Marija Zafirova Gjorgievska

Center for Drug Quality Control, Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Majka Tereza 47, 1000 Skopje, Republic of North Macedonia

Introduction

Dry powder inhalers (DPIs) are widely used for the treatment of asthma and chronic obstructive pulmonary disease, often as combination products containing a β2-adrenoceptor agonist and a corticosteroid to provide rapid and sustained therapeutic effects. Among these, formulations comprising formoterol fumarate (FF), a long-acting β2-agonist, and fluticasone propionate (FP), a second-generation trifluorinated corticosteroid, have demonstrated significant synergistic therapeutic benefits (Rajesh et al., 2020). Reversed-phase high-performance liquid chromatography (RP-HPLC) is commonly employed for the simultaneous determination of FF and FP in inhalation products (Patodkar et al., 2026), where achievement of the highest separation efficiency and quick delivery of results are key for enhancing productivity and reducing analysis cost.

This study evaluates the performance of two different generations of octadecylsilica (C18) chromatographic columns for the quantification of these active substances in DPI capsules under identical chromatographic conditions, focusing on separation efficiency and analysis time.

Materials and methods

Materials

Chemical reference standards of FF dihydrate and FP were obtained from the Ph. Eur. Acetonitrile and methanol (HPLC isocratic grade) were purchased from Carlo Erba Reagents, France, and $NH_4H_2PO_4$ (for analysis) and o-H_3PO_4 85% HPLC from Merck, Germany. Water for chromatography was obtained with a TKA-LAB Reinstwasser system (Germany).

Chromatographic conditions

Determination was performed on Agilent 1260 Infinity LC system, equipped with a diode array detector, at 239 nm, using Zorbax SB-C18 250 mm x 4.6 mm; 5 µm (Agilent Technologies) and Spherisorb ODS1, 250 mm x 4,6 mm, 5 µm (Waters Corporation) with an isocratic mobile phase consisting of buffer (1,15 g/L $NH_4H_2PO_4$ in water), acetonitrile and methanol (35:15:50, *v/v/v*) at pH 4.0. Flow rate was 1.2 mL/min, and column temperature was held at 40 °C.

Preparation of standard solutions

Standard solution of FF dihydrate and FP in methanol : water (65:35, *v/v*) in final concentrations of 0.016 mg/mL, was prepared.

*gabrielapetrovska@ff.ukim.edu.mk

Results and discussion

Under identical chromatographic conditions, elution of FP remained similar on both columns (~ 10 min), while for FF, chromatographic behavior drastically shifted, with retention time increasing from approximately 4 min on Spherisorb ODS1 to over 60 min on Zorbax SB-C18, resulting in inversion of the elution order. Peak symmetry and efficiency remained suitable for both analytes on both columns. FP is an un-ionized, highly lipophilic corticosteroid and largely unaffected by mobile phase pH, resulting in consistent chromatographic behavior across both columns. FF is a basic compound with pKa values in the range of 8–10 (AstraZeneca, 2023), characterized by relatively high polarity and moderate lipophilicity and typically exhibits RP-HPLC behavior with high affinity for stationary phases, making it sensitive to pH, buffer strength and organic modifiers (Tashkin, 2020).

Zorbax SB-C18 is built on ultra-pure, low-acidity, Type B silica, that is intentionally not end-capped in order to withstand low pH conditions through steric protection provided by diisobutyl groups. This column promotes dominant hydrophobic interactions, resulting in improved peak symmetry and reproducibility for the analysis of basic compounds. At pH 4.0, FF being predominantly protonated, would engage in strong electrostatic interactions with the partially ionized free silanol groups on the surface of the Zorbax SB-C18. Simultaneously, the bulky and highly hydrophobic aromatic rings of the FF molecule (National Center for Biotechnology Information, 2026) promote strong hydrophobic interactions with the C18 chains, further anchoring the molecule to the stationary phase.

In contrast, Spherisorb ODS1 is a classic, non-endcapped RP-HPLC column built on first-generation, Type A silica, with lower ligand density and carbon load (6.2%) and higher residual silanol activity, exhibiting weaker hydrophobic retention and greater contribution of polar and ionic interactions. Under the same conditions, on a column that has a low carbon loading, the predominantly ionized FF molecule exhibited reduced interaction with the stationary phase and greater affinity for the organic-rich mobile phase, leading to rapid elution.

Conclusion

The results demonstrate that suitable differences in C18 column chemistry, in combination with mobile phase composition and analyte ionization state, critically influence chromatographic behavior and method performance for basic pharmaceutical compounds such as FF. Although the Spherisorb ODS1 column exhibits higher silanol activity and lower chemical stability compared to more advanced C18 phases, like Zorbax SB-C18, it resulted as more appropriate for the determination of FF and FP in DPI capsules and provided satisfactory separation with acceptable analysis time for the target analytes.

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