

Separation of 5-fluorouracil and its derivatives using a stable bonded phase HILIC column

TSKgel
APPLICATION NOTE

Introduction

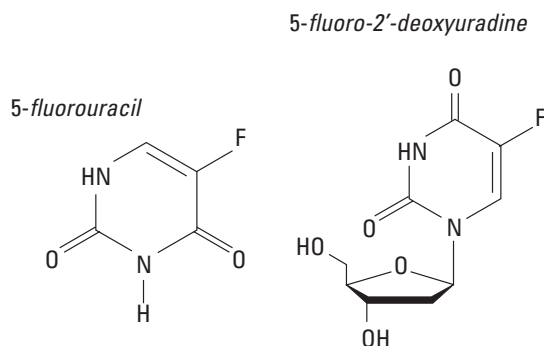
Chemotherapy agent 5-fluorouracil (5-FU) is a pyrimidine analog that has been used as an anticancer drug for more than 40 years. This compound, as well as 5-fluoro-2'-deoxyuridine, principally inhibits DNA synthesis by prior inhibition of thymidylate synthase inhibitor¹.

5-FU is highly polar and elutes in the void volume when separated by widely used reversed phase chromatography (RPC) columns, even in 100% aqueous mobile phase. The polar compounds can be retained, however, by hydrophilic interaction chromatography (HILIC) by using an amide or amino bonded phase column. The HILIC mode of separation offers ~10-fold increase in the sensitivity of detection when used in conjunction with electrospray-ionization mass spectrometry (ESIMS).

This application note discusses the effectiveness of a TSKgel NH₂-100 HILIC column in retaining both 5-FU and 5-fluoro-2'-deoxyuridine and allowing for their separation from several derivatives using a conventional HPLC system. TSKgel NH₂-100 columns are packed with spherical 3 μm silica particles containing 100 Å pores. The internal and external surfaces of the particles are derivatized with a proprietary alkylamino silane reagent, while remaining and accessible silanol groups are endcapped with trimethylsilane. This novel bonding strategy provides expanded selectivity and a better safeguard against hydrolysis of the underlying silica. Due to a high ligand density and large surface area, these columns show stronger retention of polar compounds.

Experimental Conditions

Column:	TSKgel NH ₂ -100, 3 μm, 2.0 mm ID x 5 cm
Mobile phase:	80% acetonitrile and 20% 5 mmol/L ammonium acetate in water (Isocratic)
Flow rate:	0.1 mL/min
Temperature:	ambient
Injection vol.:	10 μL
Sample:	5-fluorouracil (Sigma) 5-fluoro-2'-deoxyuridine (Sigma)



¹Dawson, R. M. C., et al., Data for Biochemical Research, 3rd ed., p. 267., Oxford University, Press, New York, (1986).

Results and Discussion

Compared to a competitive HILIC column, the TSKgel NH₂-100 column resulted in higher resolution for the analysis of 5-fluorouracil (Figures 1 and 2). The analysis with the TSKgel NH₂-100 column was consistent in retention time, capacity factor, area, asymmetry, and column efficiency with low %RSD values. As shown in Figure 3, the TSKgel NH₂-100 column was also successfully used for the analysis of 5-fluoro-2'-deoxyuridine, another polar compound in the metabolic pathway of 5-FU.

Figure 1. Separation of 5-fluorouracil using a TSKgel NH₂-100 column

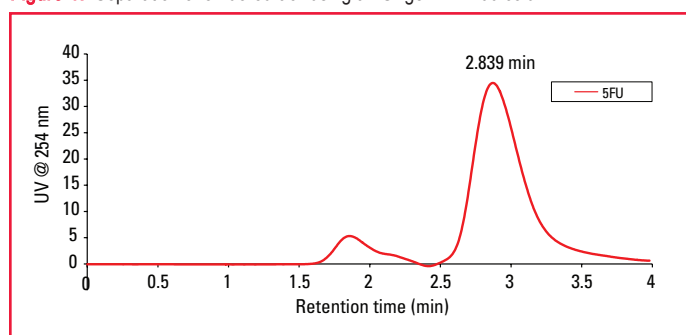


Figure 2. Separation of 5-fluorouracil using a competitive HILIC column

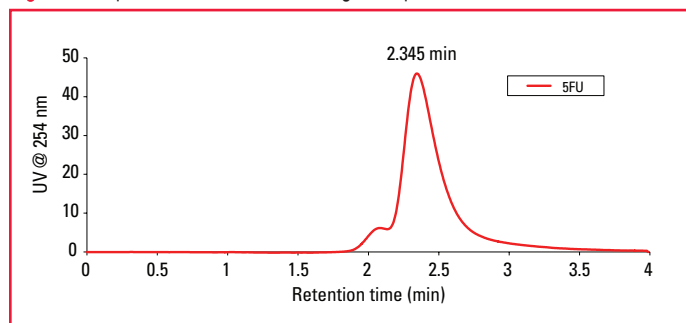
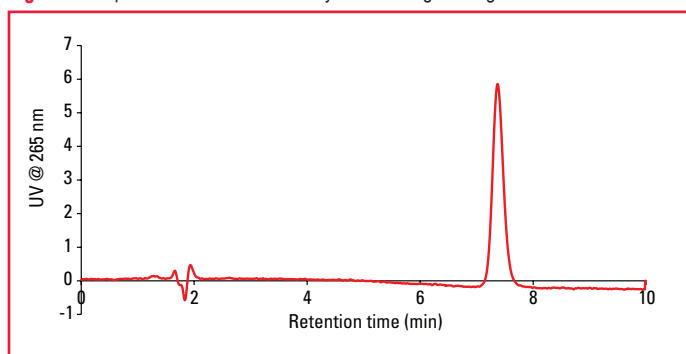


Figure 3. Separation of 5-fluoro-2'-deoxyuridine using a TSKgel NH₂-100 column



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Along with a system suitability study to determine the limit of detection and limit of quantitation, a column lifetime study was also conducted (data not shown). The TSKgel NH₂-100 column proved to be highly stable over 1000 injections without any significant change in the capacity factor of the column. The bonded phase of the TSKgel NH₂-100 columns offers higher chemical stability, a pre-requisite for reproducible results.

Conclusions

TSKgel NH₂-100 columns provide excellent separation of polar molecules, such as 5-fluorouracil and 5-fluoro-2'-deoxyuridine, with high resolution and consistency. This HILIC column yielded better separation compared to a competitive column.



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