

Evaluation of the Selectivity of Novel Stationary Phases for Supercritical Fluid Chromatography



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INTRODUCTION

Packed column supercritical fluid chromatography (pSFC) has recently seen a renewed interest in the pharmaceutical industry – in part due to the advantageous high separation efficiencies which are achievable in short analysis times. Moreover, fine tuning of selectivity is possible via mobile phase composition, and robust (generic) methodology can be utilized, which is applicable to a wide range of compounds. The complementary nature (orthogonality) of pSFC to reversed phase LC (RP-LC) is also being continuously enhanced through the introduction of new stationary phases, which is increasing the selectivity ‘space’ that pSFC operates within. Furthermore, functionalities are incorporated in the stationary phases so that the use of prep SFC-scale level offers then the advantage that only the organic modifier has to be evaporated to obtain pure product.

EXPERIMENTAL

A number of commercially available (e.g. 2-ethylpyridine from Princeton Inc. and Zorbax SB cyano from Agilent), prototype (Zymor, Inc.), and home synthesized stationary phases obtained via click chemistry have been evaluated. The prototype phases are based on incorporation of morpholine, piperazine carbamate, piperazine urea, benzamide, pyridine, pyridine/diol, and pyridine/MONOL groups in the stationary phase. The home synthesized phases include a number of molecules (e.g. pyridine, testosterone, estradiol, etc.) incorporated into the stationary phases.

A 22 component test mixture containing acidic, basic, and neutral pharmaceutical compounds was analyzed with pure methanol and methanol containing 20 mM ammonium formate as the modifiers. The latter was carried out for hyphenation with mass spectrometry.

Separation Conditions: Injection volume 5 μ L, flow rate 2.0 mL/min, outlet pressure 100 bar, detection 254nm, column temperature 40°C, and CO₂ was used as the mobile phase and methanol with and without additive was the modifier. The gradient was isocratic at 5% modifier for 1 min, then increased linearly to 40% modifier at 2%/min.

RESULTS AND DISCUSSION

I. The objective of evaluating new phases in SFC is to obtain similar results as for a 2-ethylpyridine, but without acidic/basic additives.

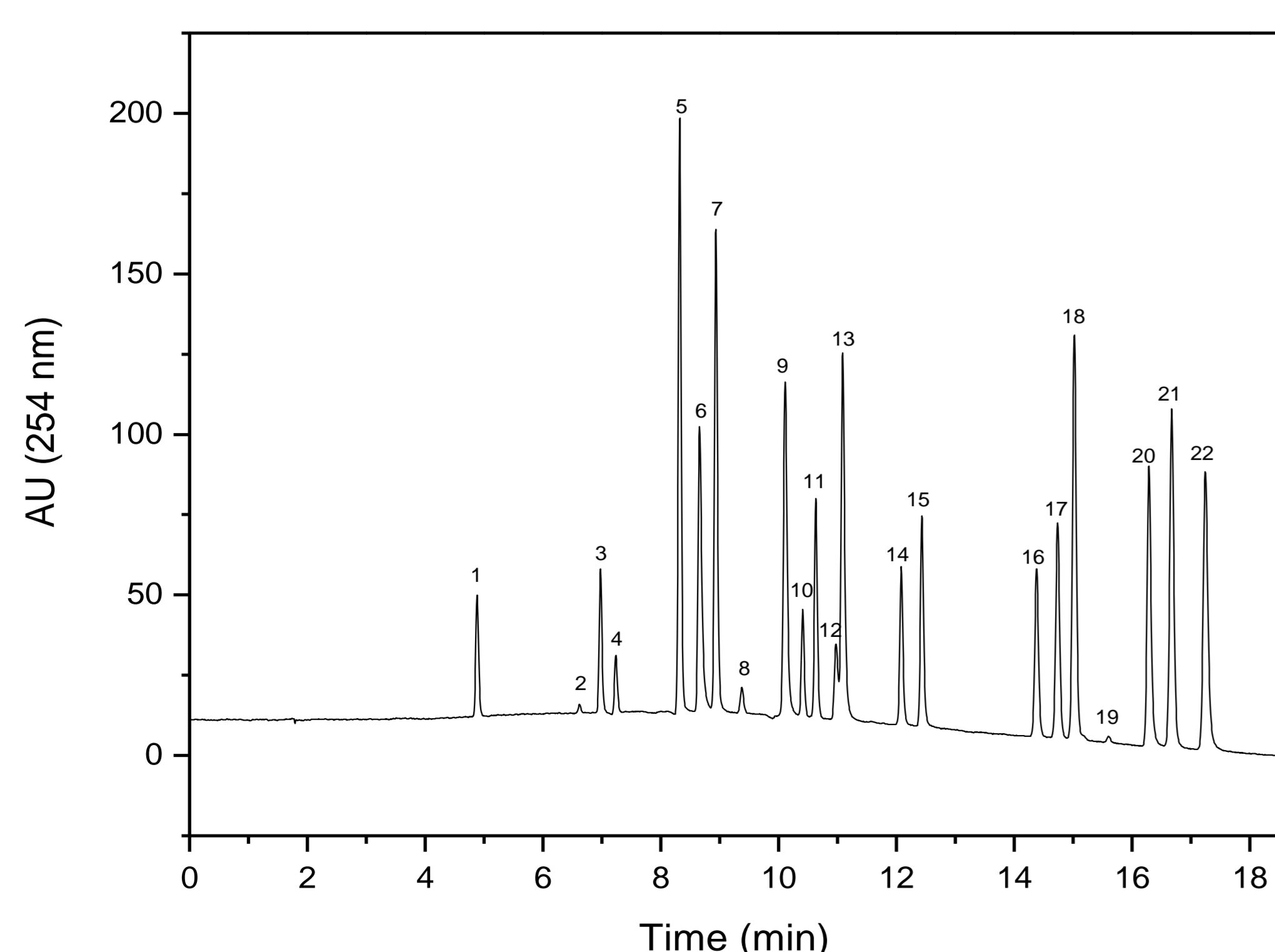


Figure 1: Chromatogram of the separation of the 22 component test mixture on the Princeton pyridine column (4.6 x 250 mm, 3 μ m particle). The modifier was methanol with 20 mM ammonium formate. All other conditions were the same as in the experimental

Caffeine (1)
Ibuprofen (2)
Theophylline (3)
Theobromine (4)
Thymine (5)
Adenine (6)
Uracil (7)
Fenoprofen (8)
Flurbiprofen (9)
Cortisone (10)
Prednisone (11)
Cytosine (12)
Hypoxanthine (13)
Hydrocortisone (14)
Prednisolone (15)
Sulfamerazine (16)
Sulfamethoxazole (17)
Sulfadimethoxime (18)
Estril (19)
Sulfaguanidine (20)
Sulfaquinoxaline (21)
Sulfamethizole (22)

II. Evaluation of all columns mentioned in the experimental section showed that:

- The Zymor pyridine column performed very well without additives (Figure 2).

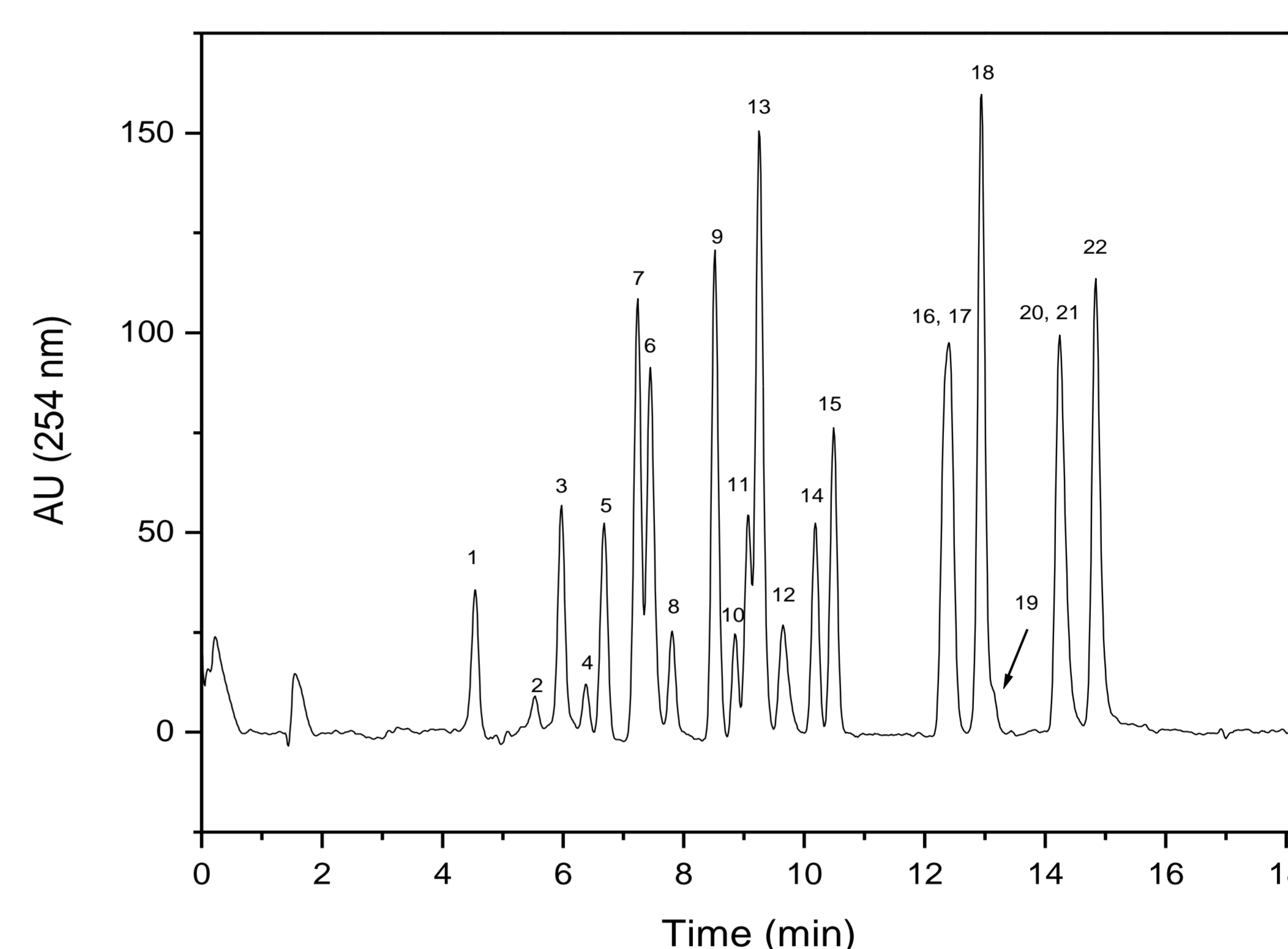


Figure 2: Chromatogram of the separation of the 22 component test mixture on the Zymor pyridine column (4.6 x 150 mm, 5 μ m particle) using pure methanol as the modifier. Separation conditions were as stated in the experimental.

- Other good phases to use without additives are:
 - Pyridine/diol, pyridine/MONOL, cyano, testosterone
- Phases that need additives to guarantee symmetry factors of > 0.9 are:
 - Piperazine urea, piperazine carbamate, benzamide, morpholine, Princeton pyridine

III. Orthogonality of the phases was determined through PCA analysis and the results are shown in Figure 3.

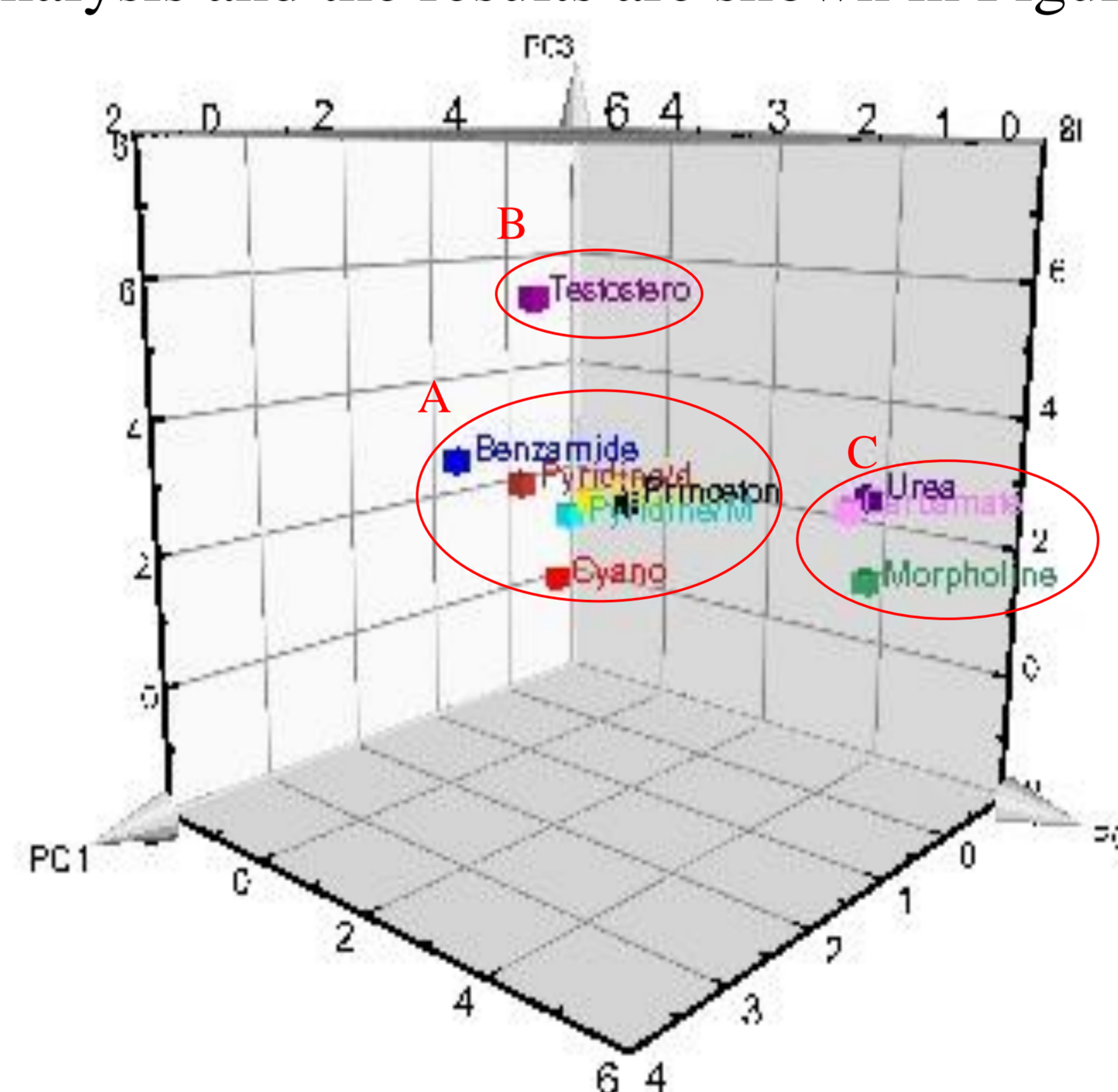


Figure 3: PCA plot of the different columns analyzed constructed using the average retention factors for all compounds.

- The consequences are:
 - For group A, the Zymor pyridine column should be selected as other phases are very similar in polarity/selectivity
 - Testosterone (Group B) has unique properties and deserved further evaluation
 - From Group C, morpholine is the most promising considering the asymmetry factors.

CONCLUSION

- Tailoring SFC phases allows the operation without additives. This opens new possibilities for prep-SFC
- 2-ethylpyridine is by far the most interesting SFC stationary phase.