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Optimize the Separation of Fluorinated Amphiles Using High-Performance Liquid Chromatography

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Abstract

Using the set of fluorinated amphiles that contain the same fluorocarbon moiety but differ in their fluorine content percentage F% (25–45%), the optimal condition for a F%-based separation of these analytes using reverse-phase chromatography was explored. It is found that optimal separation can be achieved by pairing a regular reverse-phase column (such as C8) with a fluorinated eluent (such as trifluoroethanol). Separation is further improved at higher chromatographic temperature with baseline separation achieved at 45°C. This result indicates that the separation of fluorocarbon-tagged molecules can be based on the fluorine content percentage rather than the number of fluorine atoms.

Keywords

reverse-phase chromatography; HPLC; fluorinated amphiles; fluorous; hetero-pairing; homo-pairing

1. Introduction

Fluorous mixture synthesis (FMS) is a valuable strategy for the efficient construction of chemical libraries [1]. In conventional FMS, a set of starting materials is tagged with fluorocarbons of different sizes. After the chemical transformation is completed, fluorous

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chromatography is used in the “demixing” stage to separate the fluorocarbon-tagged molecules. Chromatographic demixing relies on the difference in the number of fluorine atoms in the fluorocarbon tags [2]. Recently, we expanded FMS from the synthesis of small molecules to the synthesis of fluorinated dendrimers [3]. Alongside this expansion of synthesis scope, we demonstrated that it is possible to use the same fluorocarbon tag to separate a mixture of fluorinated molecules provided the fluorine content percentage, F%, varies across the analyte population. Hence the key enabling factor in the chromatographic separation of fluorinated analytes is not the number of fluorine atoms, but rather the fluorine content percentage.

In the aforementioned work on FMS of fluorinated dendrimers, demixing was achieved with either fluorocarbon reverse-phase or normal-phase columns [3], but not with regular hydrocarbon reverse-phase columns that contain alkyl chains such as n-C₈H₁₇ or n-C₁₈H₃₇ (C8 or C18 column). However, reverse-phase chromatography with C8 and C18 columns is the most commonly used method in HPLC. Hence it is highly desirable to achieve F%-based separation using C8 or C18 columns. This is the main objective of the present work.

In a previous study on the separation of amino acids and proteins (non-fluorinated) [4], we found that optimal analyte separation is achieved by pairing a fluorinated column (F-column) with a hydrogenated eluent (H-eluent) or a hydrogenated column (H-column) with a fluorinated eluent (F-eluent), i.e., hetero-pairing of the column and the eluent. Fluorinated eluents have been reported to induce unique selectivity on nonfluorinated analytes, which was attributed to the adsorbed solvent molecules, 2, 2, 2- trifluoroethanol, on the stationary phase surface [5]. This result suggests that it might be possible to achieve F%-based separation of fluorinated analytes by pairing a regular reverse-phase column, such as C8, with a fluorinated eluent, such as trifluoroethanol. Another useful parameter in chromatographic separation is temperature [6], which is also explored in this project.

2. Experimental Design

2.1. Design of Fluorinated Analytes

To explore F%-based chromatographic separation of fluorinated analytes, we designed a series of fluorinated amphiles, compounds **1** to **4** in Table 1. Each fluorinated amphile comprises a hydrophobic perfluorooctyl chain and a hydrophilic oligooxyethylene chain. The two parts are connected through a benzene ring (Table 1). These fluorinated amphiles contain the same perfluorooctyl chain, (n-C₈F₁₇), but differ in the length of the oligooxyethylene chain, with the repeated number varying from 4 to 16. As a result, the F% decreased steadily from *ca.* 45% in compound **1** to *ca.* 25% in compound **4**, even though the number of fluorine atoms is 17 in all the amphiles (Table 1). Also shown in Table 1 are the hydrophilicity order of the compounds, which increases from **1** to **4**, and the fluorophilicity order, which decreases from **1** to **4**. The synthesis of these compounds has been presented in our previous paper [7].

2.2. Selection of HPLC columns

To investigate how column fluorination affects the retention and separation of fluorinated analytes, a fluorinated column (FluoroFlash, purchased from Fluorous Technologies LLC)

whose stationary phase also contains a perfluorooctyl chain (n-C₈F₁₇), the same as in the analytes, was chosen. Correspondingly, the hydrogenated column contains an octyl chain (n-C₈H₁₇, Zorbax XBD-C8). The F- and H-columns have the same dimension (4.6 mm × 150 mm) to facilitate comparison.

2.3. Selection of eluents

To investigate how column-eluent pairing affects analyte retention and separation, a fluorinated solvent, trifluoroethanol (CF₃CH₂OH, TFE), and its hydrogenated counterpart, ethanol (CH₃CH₂OH, EtOH), were used as the eluents. It is noteworthy that, as a result of the strong electron-withdrawing ability of the trifluoromethyl group, TFE, with a dielectric constant of 27.68, is slightly more polar than EtOH, which has a dielectric constant of 25.30.

3. Results and discussion

We explored a total of 12 chromatographic conditions (2 columns × 2 eluents × 3 temperatures) for the separation of a mixture of compounds **1–4**. The chromatograms for each condition are presented in the Supporting Information (SI). The retention time of each analyte under each chromatographic condition is listed in Table S1. Figure 1 presents the chromatograms at 45°C.

From the data, it is clear that the elution order of the 4 analytes is determined by the eluent; analytes with higher eluent-philicity are eluted earlier than analytes with lower eluent-philicity. Specifically, when the eluent is fluorinated (TFE), the elution order matches the fluorophilicity order (**1** first, **2** second, **3** third and **4** fourth), for both F- and H-columns (Figure 1); when the eluent is hydrogenated (EtOH), the elution order matches the hydrophilicity order (**4** first, **3** second, **2** third and **1** fourth), for both F- and H-columns (Table 1 & Figure 1).

The elution order in chromatography is usually determined by the column, not the eluent. For example, in our previous work on fluorinated dendrimers, normal- and reverse-phase HPLC produce opposite elution orders [3a]. But here, the elution order is reversed by the switch of the eluent, regardless of the column. This is a remarkable result considering that the two eluents have comparable polarity as judged by the dielectric constant. This result demonstrates the uniqueness of fluorous chromatography.

Elution order notwithstanding, the effectiveness of a chromatographic condition is measured by its ability to retain and separate the analytes. The retention of an analyte is given by its retention time t_R while the separation of a pair of analytes X and Y (X eluted after Y) is given by the separation factor $\alpha_{X/Y}$, defined as:

$$\alpha_{X/Y} = \frac{t_R(X)}{t_R(Y)} \geq 1 \quad (1)$$

The individual retention times are listed in Table S1 of the Supporting Information while the separation factors for adjacent analyte pairs are presented in Table 2. The general trend is

that, at a given temperature, hetero-pairing of column and eluent leads to larger retention time for a given analyte (Table S1) and larger separation factor for a given analyte pair (Table 2).

While the retention time t_R and the separation factor α_{XY} provide accurate description of the ability of a chromatographic method to retain an analyte or to separate an analyte pair, they fall short on describing the ability of a chromatographic method to retain and separate a set of closely related analytes. To this end, we resort to statistical descriptors of retention and separation, as outlined in our previous publication [4a].

The ability of a chromatographic method to retain a set of analytes is given by the retention time mean $\mu(t_R)$, defined as:

$$\mu(t_R) = \frac{\sum_i t_{R,i}}{n} \quad (2)$$

where $t_{R,i}$ is retention time of the i -th analyte and n is the number of analytes in the set ($n = 4$ in this case). $\mu(t_R)$ for the eight chromatographic methods are listed in Table 2. It is clear that at a given temperature, hetero-pairing of column and elution solvent leads to longer retention of the entire set of analytes.

The ability of a chromatographic method to separate a set of analytes is given by the retention time variance $\sigma^2(t_R)$, defined as:

$$\sigma^2(t_R) = \frac{\sum_i [(t_{R,i} - \mu(t_R))]^2}{n - 1} \quad (3)$$

$\sigma^2(t_R)$ for the eight chromatographic methods are listed in Table 2. Similar to retention, at a given temperature, hetero-pairing of column and elution solvent leads to better separation of the entire set of analytes.

Compared with individual retention time and separation factor, the retention time mean and variance provide a better guidance when selecting a chromatographic method for a group of closely related analytes, which can be important for applications such as FMS.

While hetero-pairing of column and elute improves both retention and separation, temperature has the opposite effect on retention and separation; higher temperature reduces analyte retention but improves analyte separation. In fact, near baseline separation of the 4 analytes was achieved only at 45°C with hetero-pairing (Figure 2). Such temperature-dependent chromatographic behavior is hardly surprising since the interaction between fluorinated compounds is known to be sensitive to temperatures [8].

4. Conclusion

In this work, the chromatographic behavior of a set of moderately fluorinated analytes (*ca.* 25–45 F%) with the same fluorocarbon moiety was investigated. The elution order of the analytes corresponds to their eluent-philicity. Hydrophilic analytes were eluted first when

hydrogenated eluent was used while fluorophilic analytes were eluted first when fluorinated eluent was used. Optimal separation was achieved through hetero-pairing of column and eluent, i.e., fluorinated column with hydrogenated eluent or hydrogenated column with fluorinated eluent at elevated temperature.

5. Experimental Section

5.1 Materials and Methods

5.1.1. Fluorinated amphiles—All the analytes, **1–4**, were synthesized following our previously reported procedures [7]. The molecular weight was verified by mass spectrometry (see Supporting Information).

5.1.2. Eluents—Ethanol (EtOH) and trifluoroethanol (TFE) were purchased from Sigma-Aldrich (spectrophotometric grade). Trifluoroacetic acid (TFA) was purchased from Sigma-Aldrich.

5.1.2. Instrumentation—All the HPLC chromatograms were collected using an Agilent Technologies HP1200 Series Liquid Chromatography System housed in a temperature-controlled room.

ESI mass spectra were recorded on a Shimadzu Biotech Axima Performance System.

For columns used in this study, the fluorocarbon one is a FluoroFlash column (4.6 mm × 150 mm) while the hydrocarbon one is a Zorbax XDB-C8 column (4.6 mm × 150 mm).

5.2. Chromatographic conditions

The chromatographic conditions were: eluent A: 0.1% TFA in water; eluent B: 0.1% TFA in EtOH or TFE; gradient: 2%B/min, 60%B–100%B in 20 min; flow rate: 1 mL/min; column chamber temperature: 5°C, 25°C and 45°C; detection: UV-Vis absorption at 210 nm. For chromatographic runs at 5°C, the room temperature was set at 5°C. For chromatographic runs at 25°C and 45°C, the room temperature was set at 25°C. The retention time of each compound was obtained by a single HPLC run of the solution of each pure compound. The co-injection of all four compounds was carried out after mixing the solution of all four compounds. Peak assignment was confirmed by the single peak growth caused by the addition of each analyte to the mixture of all four analytes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- The elution order of the analytes corresponds to their eluent-philicity.
- Optimal separation was achieved through hetero-pairing of column and eluent.
- Separation is further improved by elevated temperature.
- Separation in fluorous mixture synthesis is based on the fluorine content percentage F% rather than the number of fluorine atoms.

Figure 1.

The eluent effect on elution order. **(a):** F-eluent/H-column, **(b):** F-eluent/F-column, **(c):** H-eluent/F-column, **(d):** H-eluent/H-column; chromatography temperature: 45 °C)

Figure 2.

The effect of chromatographic temperature on retention time and separation efficiency. (a: 5 °C, b: 25 °C, c: 45 °C; a, b, and c using an H-column/H-eluent pair; d using an H-column /F-eluent pair at 45 °C).

Table 1

List of Analytes

Compound no.	k	Formula	M.W. (Da)	F%	Hydrophilicity order	Fluorophilicity order
1	4	C ₂₃ H ₂₇ F ₁₇ O ₅	730.45	44.22%		
2	8	C ₃₃ H ₄₃ F ₁₇ O ₉	906.66	35.62%		
3	12	C ₄₁ H ₅₉ F ₁₇ O ₁₃	1082.87	29.83%		
4	16	C ₄₉ H ₇₅ F ₁₇ O ₁₇	1259.08	25.65%		

Table 2

Retention and Separation Descriptors of Chromatographic Methods *

Parameters	5°C				25°C				45°C			
	Homo-pairing		Hetero-pairing		Homo-pairing		Hetero-pairing		Homo-pairing		Hetero-pairing	
	F _{-Col} F _{-Sol}	H _{-Col} H _{-Sol}	F _{-Col} H _{-Sol}	H _{-Col} H _{-Sol}	F _{-Col} F _{-Sol}	H _{-Col} H _{-Sol}	F _{-Col} H _{-Sol}	H _{-Col} F _{-Sol}	F _{-Col} F _{-Sol}	H _{-Col} H _{-Sol}	F _{-Col} H _{-Sol}	H _{-Col} F _{-Sol}
Separation factor $\alpha_{1/2}$	1.05	1.00	1.05	1.06	1.03	1.05	1.06	1.08	1.03	1.05	1.07	1.08
Separation factor $\alpha_{2/3}$	1.00	1.00	1.00	1.04	1.02	1.04	1.04	1.05	1.02	1.04	1.05	1.06
Separation factor $\alpha_{3/4}$	1.00	1.00	1.00	1.03	1.02	1.00	1.03	1.04	1.02	1.03	1.04	1.05
Mean value $\mu(\text{min})$	11.60	8.11	14.60	14.93	9.97	8.58	12.70	13.07	8.68	7.62	11.11	12.42
Variance $\sigma^2(\text{min}^2)$	0.07	0.00	0.11	0.69	0.07	0.14	0.49	0.85	0.06	0.16	0.61	0.93

* Solvents and columns: fluorocarbon column (F_{-Col}, n-C₈F₁₇), hydrocarbon column (H_{-Col}, n-C₈H₁₇), fluorinated solvent (F_{-Sol}, CF₃CH₂OH), hydrogenated solvent (H_{-Sol}, CH₃CH₂OH).