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Short communication

Optimize the separation of fluorinated amphiles using high-performance liquid chromatography

Guiquan Xia [a,1], Yuqi Li [b,1], Yu Li [a], Xuefei Li [a], Hua Zhang [a], Yihua Bruce Yu [b], Zhong-Xing Jiang [a,c,*]

[a] Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (Wuhan University), Ministry of Education, and Wuhan University School of Pharmaceutical Sciences, Wuhan 430071, PR China
[b] Department of Pharmaceutical Sciences, University of Maryland, Baltimore, MD 21201, United States
[c] Key Laboratory of Organofluorine Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, PR China

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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.

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 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*-C_8H_{17} or *n*-$C_{18}H_{37}$ (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.

* Corresponding author at: Corresponding author at: Wuhan University, School of Pharmaceutical Sciences, Wuhan 430071, Hubei, PR China. Tel.: +86 27 68759220.
E-mail addresses: zxjiang@whu.edu.cn, zxjiangsioc@hotmail.com (Z.-X. Jiang).
[1] These two authors contributed equally to this work.

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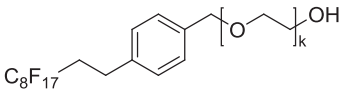
0022-1139/

Table 1
List of Analytes



Compound no.	*k*	Formula	M.W. (Da)	*F*%	Hydrophilicity order	Fluorophilicity order
1	4	$C_{25}H_{27}F_{17}O_5$	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		

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*-C_8F_{17}), 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*-C_8F_{17}), the same as in the analytes, was chosen. Correspondingly, the hydrogenated column contains an octyl chain (*n*-C_8H_{17}, 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 (CF_3CH_2OH, TFE), and its hydrogenated counterpart, ethanol (CH_3CH_2OH, 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. Fig. 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 (Fig. 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 and Fig. 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:

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

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

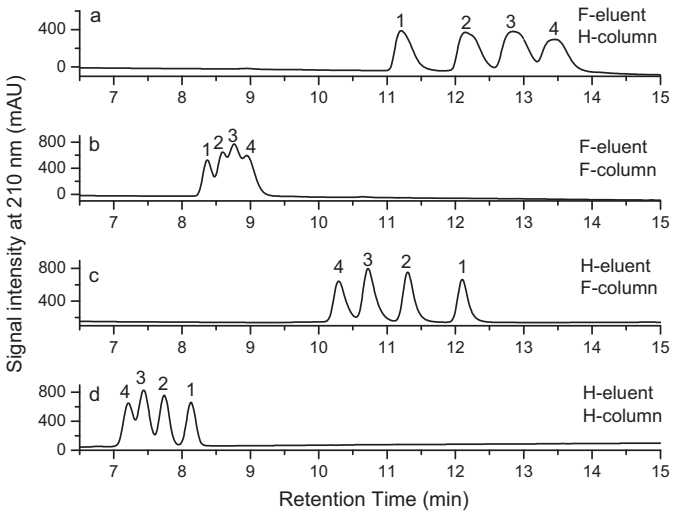


Fig. 1. The eluent effect on elution order. ((a) F-eluent/H-column, (b) F-eluent/F-column, (c) H-eluent/F-column, (d) H-eluent/H-column; chromatography temperature: 45 °C).