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Application of partially fluorinated carboxylic acids as ion-pairing reagents in LC/ESI-MS

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ABSTRACT

This report describes the application of partially fluorinated carboxylic acids as ion-pairing reagents for basic analytes in high-performance liquid chromatography coupled with electrospray ionization mass spectrometry (LC/ESI-MS) in positive-ion mode.

Partially fluoridated carboxylic acids such as difluoroacetic acid, 3,3,3-trifluoropropionic acid and 3,3,3-trifluoromethyl-2-trifluoromethylpropionic acid functioned as volatile paired-ion similarly as trifluoroacetic acid (TFA). These acids provided basic analytes larger retention factor (k) compared to acetic acid or formic acid in LC. The ESI-MS signal strength of analytes with these acids were higher than that of TFA and was analogous to that of acetic acid or formic acid.

The performances of partially fluorinated carboxylic acids in LC and ESI-MS for basic analytes were analyzed by multivariate statistical analysis using physicochemical descriptors of acids. Equations obtained in the analysis enabled us the quantitative evaluation of the performance of fluorinated carboxylic acids as ion-pair reagents for basic analytes in LC/ESI-MS.

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1. Introduction

Most of the active pharmaceutical ingredients are composed of basic compounds and, currently, many drug candidates have a high efficacy. High-performance liquid chromatography coupled with electrospray ionization mass spectrometry (LC/ESI-MS) is the current analytical method of choice for quantitation of active pharmaceutical ingredients and related substances in various samples. MS offers the advantages of sensitivity and selectivity, which increases throughput by combining several compounds in one sample. Unfortunately, however, LC/ESI-MS has some limitations that relate to instrument design and the ionization process [1]. The LC mobile phase additives that are applicable to MS must be volatile; otherwise, significant ESI signal suppression would occur. Therefore, volatile reagents such as acetic acid (AA), formic acid (FA), ammonium acetate and ammonium formate are usually applied to the mobile phase additives for LC/ESI-MS. These additives give sufficient sensitivity to the analytes that are being analyzed using MS; however, their ion-pairing ability [2–6] for basic drugs and peptides is low or non-existent, and often results in insufficient separation in LC and low-quality MS data. In such a case, the co-presence of other analytes in a fraction may lead to ESI signal suppression of the analyte [7], defined as ion suppression [8,9]. Therefore, improvement in separation efficiency (resolution) in LC reduces ion suppression in MS and this makes an analytical method more reliable.

In LC/ESI-MS, a reversed-phase (RP) column, such as an octadecylsilyl-silica (ODS) bead column, is commonly used. Recently developed RP columns have shown remarkable improvements in their performance due to the use of metal-free silica with advanced end-capping of residual silanol. However, these silica base columns still have some electrostatic interactions between basic analytes and silanol, which sometimes results in undesirable peak shapes. In such cases, an ion-pairing agent, such as TFA, is used as a mobile phase modifier to inhibit the unwanted electrostatic interactions between basic analytes and residual silanol.

The optimum concentration of TFA in the mobile phase is 0.2–0.25% (up to 32 mM) for separation of peptides in LC [10]; however, TFA suppress the ESI signal intensity due to ion-pair formation, high conductivity and surface tension [11,12]. Recent progress in the sensitivity of ESI-MS instruments allows us a usage of TFA at certain concentration. However, a mobile phase additive that could supersede AA, FA and TFA has, so far, not been reported from viewpoint of sensitivity in ESI-MS or separation efficiency in LC.

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Perfluorinated ion-pairing agents such as pentadecafluorooctanoic acid (PDFOA) [13–16], pentafluoropropionic acid (PFPA) [17–20], heptafluorobutyric acid (HFBA) [19,21–29]. and tridecafluoroheptanoic acid (TDFHA) [14]. were often used in ion-pair LC/ESI-MS analysis of environmental pollutants, drugs, peptides and proteins. Differentiation of these perfluorinated carboxylic acids in terms of volatility was made by quantitative investigation using evaporative light-scattering detection [30]. These perfluorinated carboxylic acids are strong ion-pairing reagents for basic analytes and work at lower concentrations than TFA in LC [19,31]. Therefore, the use of these acids at low concentration would be more useful than that achieved with TFA.

The ionization efficiency is based on the relationship between the properties of ionic groups in the target molecule and the pK_a value of the acidic mobile phase modifier [32], conductivity and surface tension of the solution [11,12].

In the present investigation, to find useful ion-pair reagents applicable to ESI-MS, we evaluated the performance of partially fluorinated carboxylic acids as ion-pair reagents for basic drugs and peptides in LC/ESI-MS. In addition, the performances of partially fluorinated carboxylic acids in LC and ESI-MS for basic analytes were analyzed by multivariate statistical analysis using the physicochemical descriptors of acids.

2. Experimental

2.1. Materials and reagents

Methanol, acetonitrile, acetic acid (99.8% purity) and formic acid (99% purity) were purchased from Wako Pure Chemical (Osaka, Japan). PFPA, HFBA, nonafluoropentanoic acid (NFPA) and PDFOA were purchased from Tokyo Kasei (Tokyo, Japan). Trifluoroacetic acid (99.8% purity) was purchased from Pierce (Rockford, IL, USA). Difluoroacetic acid (DFA, 98% purity), 3,3,3-trifluoropropionic acid (TriFPA, 98% purity), 2,2,3,3,-tetrafluoropropionic acid (TetraFPA, 96% purity), 3,3,3 trifluoro-2-(trifluoromethyl)propionic acid (TFTFPA, 97% purity), pyridoxine, atenolol, popranolol, nadolol, sulpiride, tetrahydrozoline, imipramine and angiotensin III were purchased from Aldrich (Milwaukee, WI, USA). All the reagents used in this study were used as obtained without further purification. Water was purified with a Milli-Q TOC system (Millipore, Bedford, MA, USA) prior to use.

2.2. Measurement of conductivity and pH value of acid solution

A CM60-V conductivity meter (TOA Electrochemical Measuring Instruments, Tokyo, Japan) and an HM-60V pH meter (TOA Eelectrochemical Measuring Instruments) were used to measure conductivity and pH of acid aqueous solutions at 25 °C.

2.3. Liquid chromatography coupled to mass spectrometry

For the LC/ESI-MS analysis, a LCMS-2010A single-stage quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) was equipped with two LC-10ADvp pumps, a SIL HT injector and a CTO-10Avp column oven. As an HPLC column, a silica base ODS column of Pack Pro C18 (75 mm × 2.1 mm i.d.; YMC, Kyoto, Japan) was employed. LC-10ADvp pumps delivered a mobile phase at 200 μL/mL and were applied to the ESI sprayer. Five μL of the drug mixture (40 μg/mL), peptide (angiotensin III, 10 μg/mL) or blank sample (15% methanol) were injected into the LC. Ten mM of acid in 15% methanol was used as a mobile phase for drugs in an isocratic mode. For LC/ESI-MS analysis for angiotensin III, 2.5–50 mM of acids in 20% methanol in isocratic elution was used as a mobile phase. The mass spectrometers were operated in positive-ion ESI mode and normal scans were performed with a scan speed of 10 ms/scan in the range of 150–500 amu. Data processing was carried out using LCMS Solution software, the retention factor (k) of drugs was determined from the mass chromatograms and log k was calculated for compounds ($n=3$).

2.4. Electrospray mass spectrometry

A Harvard Model 22 syringe pump (South Natick, MA, USA) was used with a flow rate of 5 μL/min. The normal scan was performed with a scan speed of 10 ms/scan in the range of 200–600 amu and the scan number was 20 ($n=3$).

Drugs (concentration; 500 ng/mL) in 30% methanol containing 10 mM of acid were infused by syringe into the mass spectrometer at a flow rate of 5 μL/min. The sample solution containing 10 mM of TFA was analyzed as a control sample and the relative signal intensity of analytes against TFA was calculated.

2.5. Multivariate statistical analysis for log k and ESI-MS intensity using physicochemical descriptors of acid

The physicochemical descriptors for carboxylic acid were calculated with Absolv in ADME box 2.0 from Pharma Algorithms, Inc. (Toronto, Canada), Daylight version 4.72 from Daylight Chemical Information Systems (CA, USA) and Pallas 3.0 from CompuDrug International (CA, USA) and tabulated in Table 2. Absolv calculated the solvent-associated properties using an Abraham-type equation [33]. The calculated descriptors in Absolv were the hydrogen-bonding acidity (A), the hydrogen-bonding basicity (B), the dipolarity/polarizability parameter (S), the excess molar refraction (E) in (cm^3/mol)/10 and the McGowan's characteristic molecular volume (V) in (cm^3/mol)/10. Daylight and Pallas calculated the Clog P and pK_a of acids, respectively. The pK_a is not a physicochemical descriptor and this parameter should have been excluded; however, the acids that were evaluated in this study were all carboxylic acids, so we used pK_a as a physicochemical

Table 1
Conductivity and pH of 10 mM of carboxylic acid solution.

Structural formula	Name (abbreviation)	Conductivity (mS/m)	pH
HCOOH	Formic acid (FA)	51	2.87
CH_3COOH	Acetic acid (AA)	16	3.33
CF_2HCOOH	Difluoroacetic acid (DFA)	309	2.07
CF_3COOH	Trifluoroacetic acid (TFA)	348	2.06
CF_3CH_2COOH	Trifluoropropionic acid (TriFPA)	98	2.55
CF_2HCF_2COOH	Tetrafluoropropionic acid (TetraFPA)	323	2.02
$C(CF_3)_2HCOOH$	3,3,3-Trifluoromethyl-2-trifluoromethyl propionic acid (TFTFPA)	315	2.05
C_2F_5COOH	Pentafluoropropionic acid (PFPA)	359	2.04
C_3F_7COOH	Heptafluorobutylic acid (HFBA)	340	2.00
C_4F_9COOH	Nonafluoropentanoic acid (NFPA)	348	2.03
$C_7F_{15}COOH$	Pentadecafluorooctanoic acid (PDFOA)	295	2.05

parameter. These physicochemical properties of the carboxylic acid are tabulated in Table 1.

The quantitative structure–retention factor relationship (QSRR) and the quantitative structure–ESI-MS intensity relationship (QSEIR) for carboxylic acids were investigated to analyze the experimental results relating to the physicochemical descriptors of the acids. In these evaluations, *E* and *V* were excluded from the calculation because of their high correlation (*r*) between Clog *P* over 0.8; pK_a, Clog *P*, *A*, *B* and *S* were used in this analysis. A multivariate statistical analysis of QSRR and QSEIR was performed using the TAHENRYOKAISEKI (MULTIVARIATE STATISTIC ANALYSIS in English) version 4.0 program (Esumi, Tokyo, Japan); the analysis led to the development of a theoretical model for prediction of the experimental results using the physicochemical descriptors of acids.

To validate the obtained theoretical model, each data set (acid species and observed data) was eliminated to perform the same prediction [leave-one-group-out analysis (LOO)] and the appropriateness of the predicted results was confirmed.

3. Results and discussion

3.1. Conductivity measurement and volatility assessment for acids

TFA works as a paired ion for basic analytes in LC; however, it causes the lowering of ESI signal intensity for analytes in ESI-MS because of its high conductivity [11,12]. To find the novel ion-pairing reagents in LC/ESI-MS, various partially fluorinated carboxylic acids, which would weaken their acidity and result in the deduction of their conductivity, were selected. The conductivity (mS/m) and pH value of their aqueous solution at a concentration of 10 mM were measured to assess the probability of the performance of these acids in LC/ESI-MS.

The results of conductivity and pH measurements for acid solutions are shown in Table 1. The pH values and conductivity were well correlated for the acids tested in this study, and partially fluorinated carboxylic acids, particularly TriFPA, showed less conductivity than TFA. As mention above, it is known that ESI-MS signal intensity is controlled by solution conductivity. The observation that TriFPA had decreased conductivity indicated that a certain structure of partially fluorinated carboxylic acids would give high ionization efficiency of analytes in ESI-MS.

3.1.1. Effect of the acid species on chromatographic retention for model compounds

In LC/ESI-MS, LC is considered to be a pretreatment process that separates or purifies the sample from the other components in sample matrices. Sufficient separation in LC enabled us to obtain satisfactory MS data—that is, either mass chromatograms or mass spectra. Hence, it is important to evaluate the effect of acids on the retention properties of analytes, which affects the performance of chromatographic separation. Retention factors (log *k*) of basic drugs in LC were determined with a mobile phase containing 10 mM of acid.

The effect of mobile phase additive on log *k* for drugs is shown in Fig. 1 and, in this evaluation, the hold-up time (t_0) was determined as 0.55 min from the chromatogram of blank sample. Every partially fluorinated acid functioned as an ion-pairing reagent for basic drugs in LC—for example, TFA gave a larger log *k* than FA or AA. The increase in the number of fluoride ions in the structure of acetic acids (AA < DFA < TFA) or propionic acids [TriFPA < TetraFPA < PFPA (< TFTFPA)] resulted in the enhancement of log *k* for analytes caused by ion-pairing efficiency. In practice, sufficient chromatographic separation for sulpiride and atenolol was not observed with FA, AA or TriFPA; however, TFTFPA separated these drugs (Fig. 1). Long-chain perfluorinated acid surfactants of HFBA and PDFOA gave analytes a large log *k*, as expected and log *k* for tetrahydrozoline and nadolol were too large to determine under the condition employed in the present investigation. The effect of partially fluorinated carboxylic acids on separation efficiency (selectivity) for compounds was analyzed by calculating Δlog *k*, which is an indicator of the structural effects of paired ions in ion-pair formation with drugs (see Eq. (1)).

$$\Delta \log\ k = \log\ k2 - \log\ k1 = \log \frac{k2}{k1} \quad (1)$$

As shown in Fig. 1, the log *k* of sulpiride and atenolol were almost equivalent; nevertheless, the structure was distinctly different. Therefore, the Δlog *k* between sulpiride and atenolol could be an indicator of the structural effect of acids in ion-pair formation.

The increase in hydrophobicity of acid (TFA < PFPA < HFBA < PDFOA) resulted in the increased Δlog *k* between sulpiride and atenolol for better separation. Meanwhile, significant differences in Δlog *k* were not observed for the drugs with DFA, TFA, TriFPA, TetraFPA and TFTFPA. This means that apparent structural effect was not observed in ion-pair formation with these acids to chemically distinguish between sulpiride and atenolol.

Partially fluorinated carboxylic acids were found to be useful ion-pairing reagents in LC/ESI-MS, as stated above. We evaluated the effect of acid concentration (2–50 mM) on log *k* of hydrophilic angiotensin III as a model peptide with FA, DFA, TFA, TriFPA and TFTFPA. The obtained results are shown in Fig. 2.

The higher the acid concentration, the larger log *k* was observed for angiotensin III with every acid except FA. The rank

Table 2
Calculated physicochemical descriptors of carboxylic acid.

Structural formula	Acid	M.W.	pK_a[a]	Clog *P*[b]	*A*[c]	*B*[c]	*S*[c]	*E*[c]	*V*[c]
HCOOH	FA	46.01	3.75	−0.54	0.44	0.38	0.59	0.28	0.32
CH_3COOH	AA	60.02	4.76	−0.19	0.62	0.45	0.64	0.22	0.46
CF_2HCOOH	DFA	96.00	1.34	0.10	0.76	0.37	0.53	0.03	0.50
CF_3COOH	TFA	113.99	0.43	0.37	0.75	0.32	0.37	−0.03	0.52
CF_3CH_2COOH	TriFPA	128.01	3.01	1.09	0.62	0.38	0.40	−0.01	0.94
CF_2HCF_2COOH	TetraFPA	146.00	−0.83	1.52	0.75	0.38	0.57	−0.13	0.68
$C(CF_3)_2HCOOH$	TFTFPA	196.00	1.35	2.38	0.66	0.30	0.29	−0.29	0.85
C_2F_5COOH	PFPA	163.99	0.51	2.51	0.75	0.34	0.33	−0.20	0.69
C_3F_7COOH	HFBA	213.99	−1.16	2.30	0.75	0.35	0.27	−0.36	0.87
C_4F_9COOH	NFPA	263.98	0.71	2.53	0.74	0.36	0.22	−0.52	1.05
$C_7F_{15}COOH$	PDFOA	413.97	−2.08	3.24	0.73	0.38	0.05	−1.02	1.57

[a] Pallsa 3.0.
[b] Daylight ver.4.72.
[c] Absolve in ADME box 2.0.

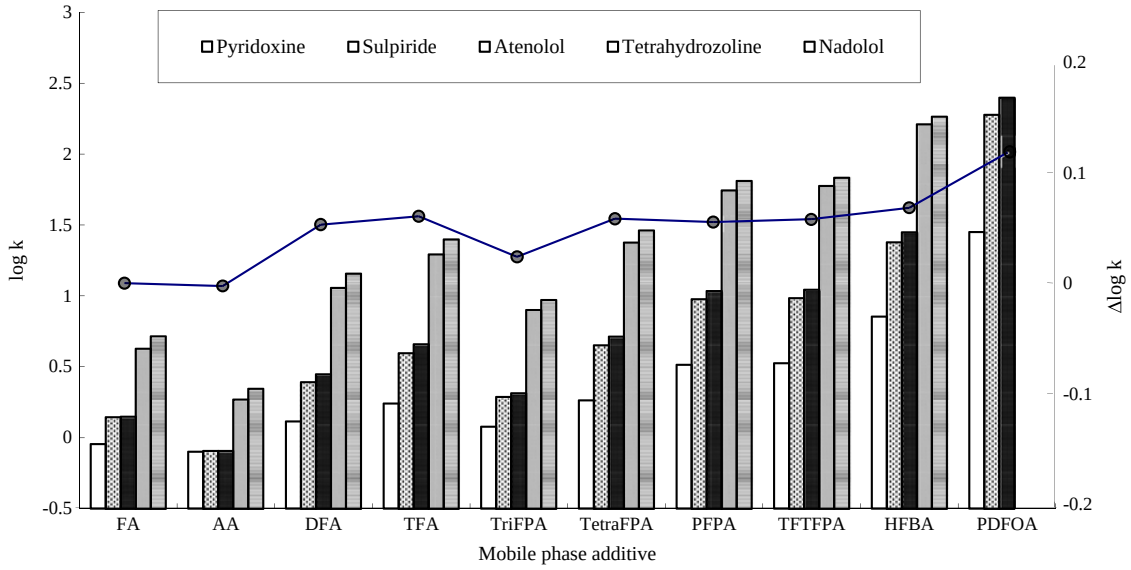

Fig. 1. Effect of mobile phase additive on log *k* of drugs. LC condition: Isocratic separation with a mobile phase of 15% methanol containing 10 mM of acid.

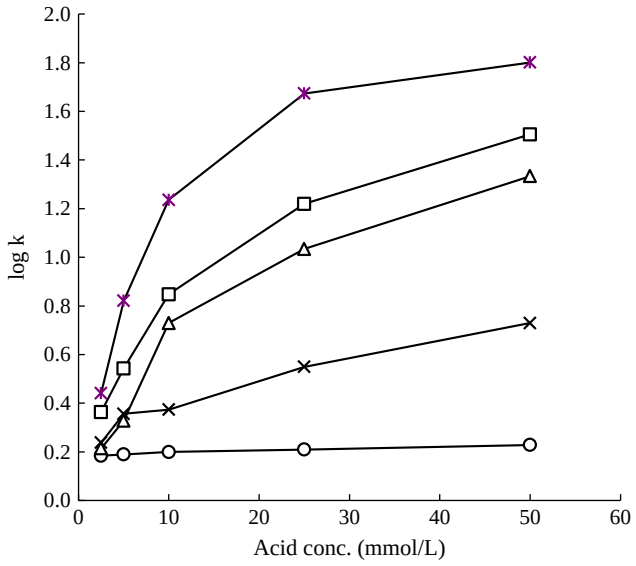

Fig. 2. Effect of mobile phase additive concentration on log *k* of angiotensin III ○FA, ×TriFPA, ΔDFA, □TFA and ✶TFTFPA.

orders of log *k* of the angiotensin III depend on the mobile phase additive species and were the same as those drugs that are shown in Fig. 1. TFTFPA gave angiotensin III a log *k* two times larger than that of TFA. For optimum resolution of peptides in LC, up to 32 mM of TFA is required [10]; however, the use of TFTFPA as a mobile phase additive in LC would offer a sufficient retention of peptides at a concentration of approximately 10 mM (Fig. 2). From a practical point of view, 10 mM of TFTFPA in the mobile phase gave analytes a large log *k* in LC; therefore, TFTFPA would be suitable in the analysis of hydrophilic, basic analytes.

3.2. Effect of the acid on ESI-MS

It was reported that the experimental conditions and employed mass spectrometer, etc., strongly influence on the ESI-MS sensitivity of analytes [34]. In the present study, to investigate the effect of acids on analytes ESI-MS sensitivity, β-blockers (pindolol, nadolol and atenolol) with various hydrophobicity and tricyclic amines of imipramine in 30% methanol containing 10 mM of each acid was investigated. As shown in Fig. 3, AA and FA gave a higher ESI signal intensity than TFA, as expected. Perfluorocarboxylic acids (TFA, PFPA, HFBA, NFPA and PDFOA) gave similar signal intensities and the apparent relationship between carbon chain length of acids and ESI signal intensity was not observed. The use of 10 mM of PDFOA might not be appropriate for this evaluation due to its relatively low volatility [30]; however, apparent defects, such as the deposition of PDFOA on the ESI interface caused by the insufficient volatility of PDFOA, were not observed in this study.

On the other hand, partially fluorinated carboxylic acids (DFA, TriFPA, TetraFPA and TFTFPA) showed a higher ESI signal intensity for drugs than they did for perfluorocarboxyic acids. Particular attention should be paid to the sufficient ESI signal intensity of DFA, TriFPA and TTFTPA, which were comparable to FA and AA. These results indicated that partially fluorinated carboxylic acid with low conductivity did not cause significant suppression of the ESI signal intensity for analytes. Furthermore, in LC/ESI-MS analysis for the drugs, similar relationships between acid species and peak areas for drugs were observed (data not shown). From these results, we concluded that partially fluorinated carboxylic acids are applicable to LC/ESI-MS for compounds with sufficient sensitivity.

3.3. Multivariate statistical analysis for log k and ESI-MS intensity with physicochemical descriptors of acids

To our knowledge, there is no report describes the analysis of QSRR and QSEIR for paired-ions including partially fluorinated carboxylic acids in LC/ESI-MS. We attempted to clarify the QSRR and QSEIR for each compound; the results of log *k* and ESI signal intensity of the compounds were analyzed by multivariate statistical analysis using the physicochemical descriptors of acids. First, to analyze the QSRR, the retention data set of pyridoxine (shown in Fig. 1) was analyzed with the five descriptors (pK_a, Clog *P*, *A*, *B* and *S*) of carboxylic acids (Table 1). These five descriptors of carboxylic acid were computed for the log *k* of five compounds by multivariate statistical analysis methodology using a validation procedure. The first multivariate statistical analysis of log *k* of the drugs led us to obtain the following equation: log *k* of drug = *a* Clog *P* + *b* pK_a + constant, where *a* and *b* are coefficients for Clog *P* and pK_a, respectively. This equation seemed to be appropriate; however, LLO analysis results revealed that the adoption of Clog *P* in the equation was not appropriate, so we carried out the analysis

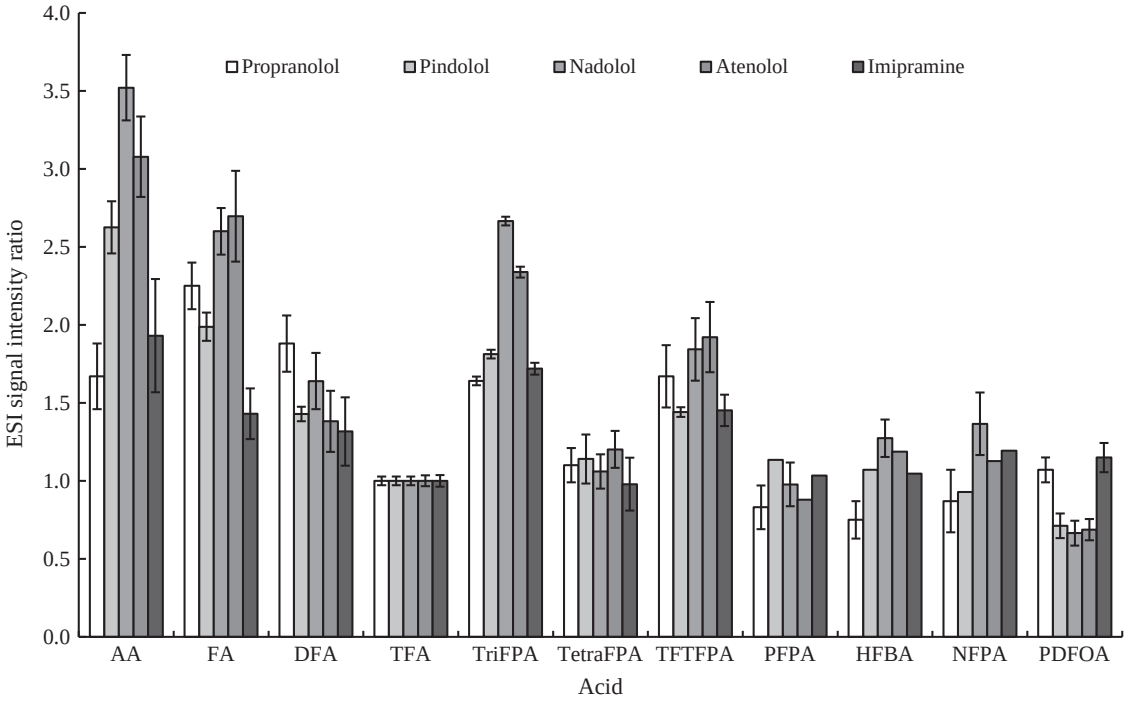

Fig. 3. Effect of acids on ESI signal intensities for drugs. Drugs (500 ng/mL) in 30% methanol containing 10 mM of acid was infused to ESI-MS and relative ESI signal intensity against TFA was calculated.

Table 3
Multiple regression results for log *k* of compounds.

Compound	Parameter	Regression coefficient	*F* value	p-Level	R^2
Pyridoxine	pK_a	−0.10	10.42	0.02	0.874
	Dipolarity/ palarizerbility	−1.74	8.56	0.03	
	Hydrogen bonding basicity	2.95	2.09	0.21	
	Constant	0.11			
Sulpiride	pK_a	−0.14	13.19	0.01	0.876
	Dipolarity/ palarizerbility	−1.54	7.71	0.03	
	Constant	1.48			
Atenolol	pK_a	−0.15	15.74	0.01	0.890
	Dipolarity/ palarizerbility	−1.58	8.35	0.03	
	Constant	1.56			
Tetrahydrozoline	pK_a	−0.19	21.75	0.00	0.918
	Dipolarity/ palarizerbility	−2.01	11.56	0.01	
	Constant	2.42			
Nadolol	pK_a	−0.19	24.53	0.00	0.923
	Dipolarity/ palarizerbility	−1.92	11.61	0.01	
	Constant	2.46			

Table 4
Multiple regression results for ESI-MS signal intensity of compounds.

Compound	Parameter	Regression coefficient	*F* value	p-Level	R^2
Propranolol	Hydrogen bonding acidity	−6.50	19.27	0.00	0.707
	Constant	6.03			
Pindolol	Hydrogen bonding acidity	−3.82	24.95	0.00	0.890
	Dipolarity/ palarizerbility	1.32	9.39	0.02	
	Constant	3.51			
Nadlol	pK_a	0.23	6.13	0.04	0.832
	Hydrogen bonding acidity	−4.07	4.20	0.08	
	Constant	4.25			
Atenolol	pK_a	0.21	6.83	0.04	0.875
	Hydrogen bonding acidity	−2.51	2.33	0.18	
	Hydrogen bonding basicity	4.83	2.27	0.18	
	Constant	1.36			
Imipramine	Hydrogen bonding acidity	−2.52	12.89	0.01	0.617
	Constant	3.03			

using the descriptors except Clog *P* and the equation for QSRR was explained as Eq. (2) with coefficients tabulated in Table 3. In the table, the *F* value for a variable indicates its statistical significance. This is a measure of the extent to which a variable makes a unique contribution to the prediction of group membership. The p-level represents the probability of error that is involved in accepting the hypothesis that the differences between the parameter estimates are equal to zero and, therefore, that they are all of equal magnitude. Coefficient of determination (R^2) indicated the coefficient of determination between the observed values and the predicted values.

$$\text{Log } k \text{ of drug} = a\text{p}K_a + bS + cB + \text{constant} \quad (2)$$

where *a*, *b* and *c* are coefficients for pK_a, *S* and *B*, respectively.

These multivariate analysis results indicated that the lower the pK_a value and the dipolarity/polarizability of acid, the larger the log *k* of the analytes in LC. A linear relationship between the experimental value and the predicted value showed a satisfactory correlation of more than 0.874 for compounds (Table 3). These equations showed that the log *k* values of each compound was regulated by the pK_a value and the dipolarity/polarizability of the paired-ions, which indicated that the ion-pairing ability of acids and the retention capability of an acid-analyte would be evident on reverse phase in LC.

The ESI signal intensity of drugs was tested using carboxylic acid as shown in Fig. 3; these results were analyzed and the following Eq. (3) with coefficients tabulated in Table 4 was obtained

$$\text{ESI} - \text{MS signal intensity of compounds} = dA + eB + fS + \text{constant} \quad (3)$$

where d, e and f are coefficients for pK_a, and S.

The multivariate analysis results for ESI-MS signal intensity indicated that the lower hydrogen-bonding acidity gave analytes high ESI-MS intensity. In addition, acids with a high pK_a have a tendency to give high ESI-MS intensity for analytes.

The linear relationship between the experimental value and the predicted value showed correlations for R^2 values of 0.617–0.890 (Table 4). LOO analysis showed that the ESI signal intensity of all compounds depended on the hydrogen-bonding activity of the acid. Conductivity or acidity of the acid controls the ESI signal intensity for analytes [11,12] and multivariate statistical analysis results confirmed that acidity of acid as a mobile phase additive controls ESI signal intensity. Thus, partially fluorinated carboxylic acids with low acidity minimally suppress ESI signal intensity. The **c**ontribution of S (dipolarity/polarizability) to ESI signal intensity was observed only for pindolol; this was probably due to the intermolecular electronic effects between the hydroxyl group and the amine on an indole ring.

The descriptors in Eqs. (2) and (3) were independent of each other and the effective parameters were also conceptually different. Therefore, unfortunately, the apparent relationship between the effect of ion-pair formation of the acid and ESI signal intensity was not observed. On the other hand, from the combined calculation results of these equations, it was possible to estimate the performance of imaginary carboxylic acids in ion-pair LC/ESI-MS. As a result, it was indicated that partially fluorinated carboxylic acids, such as CF_3–$(CF_2)_n$–CH_2–COOH ($n \geqq 1$), give large retention factors and high ESI-MS sensitivities for basic analytes in LC/ESI-MS. These partially fluorinated carboxylic acids would be promising mobile phase additives and still enhance the performance of ion-pair LC/ESI-MS for basic analytes.

4. Conclusion

We investigated the application of partially fluorinated propionic acid as a mobile phase additive in LC/ESI-MS. Partially fluorinated carboxylic acids functioned as paired-ions for basic drugs in LC and improved the ESI signal intensity in LC/MS compared with TFA. In addition, it was indicated that certain structures of partially fluorinated carboxylic acids, such as CF_3–$(CF_2)_n$–CH_2–COOH ($n \geqq 1$), would give large log k in LC and high sensitivity in ESI-MS and therefore contribute to the progress in the analytical, pharmaceutical, biomedical and biological sciences.

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