

## Technical report No.31

### UHPLC applications in 0.1% formic acid mobile phase

#### Introduction

It is necessary to consider various conditions when constructing a method. Above all, pH conditions are extremely important and have a great influence on the peak shape of the compound, so it is likely that you spend a lot of time.

In this report, we acquired the application data of UHPLC column focused on 0.1% formic acid. This application data can be readily applied to LC/MS methods.

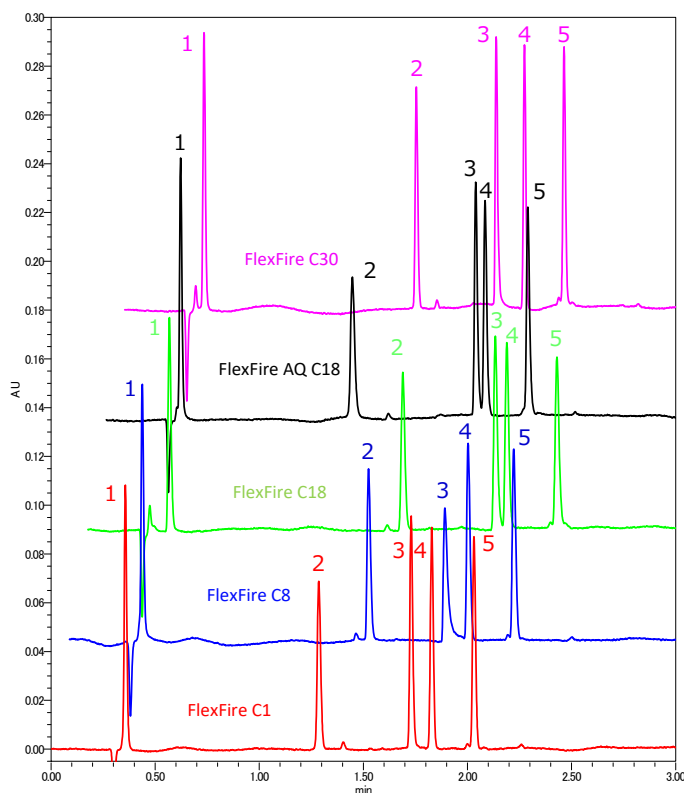
#### ■ Additives we often use

	Additive name
Acidic	Acetic acid, Formic acid
	DFA, TFA
Neutral	TEAA
Alkalinity	Ammonia water, TEA

Volatile additives are selected to accommodate many detectors.

Phosphoric acid may also be used if it is a UV detector only.

#### Analysis of low molecular weight peptides



#### Conditions:

Column: FlexFire series, 1.6um (2.0x50mm)  
 Mobile phase: A) Water+0.1%HCOOH B) Acetonitrile+0.1%HCOOH

#### Gradient:

min	mL/min	%A	%B	Curve
0.00	0.5	95	5	
1.46	0.5	75	25	6
2.93	0.5	75	25	6
2.96	0.5	95	5	6

#### Temperature:

40°C

#### Sample:

HPLC Peptide standard mixture (SIGMA)

1. Gly-Tyr
2. Val-Tyr-Val
3. Angiotensin II
4. Methionine Enkephaline
5. Leucin Enkephaline

#### Injection volume:

0.2uL

#### System:

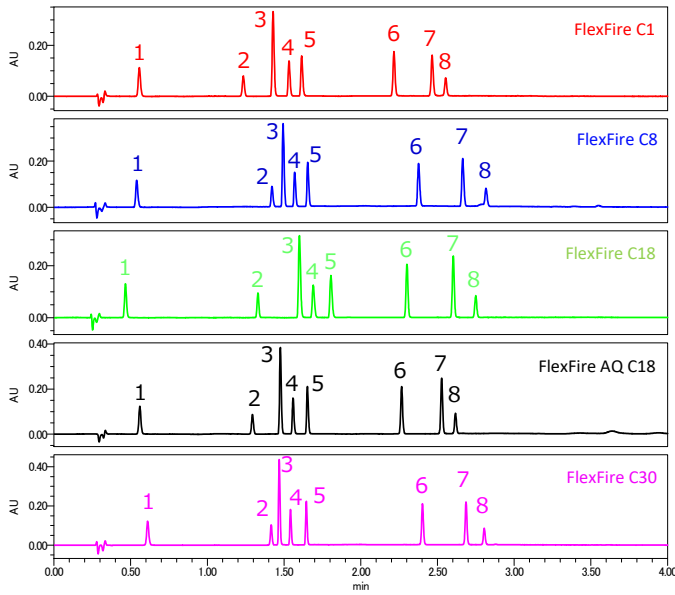
Waters ACQUITY UPLC H-Class PLUS

#### Mixer:

100uL

In some cases, low molecular weight peptides are analyzed by adding TFA to the mobile phase, but with the FlexFire series, low molecular weight peptides can be analyzed under 0.1% formic acid conditions.

## Analysis of Pharmaceutical



### Conditions;

Column: FlexFire series, 1.6um (2.0x50mm)  
 Mobile phase: A) Water+0.1%HCOOH B) Acetonitrile+0.1%HCOOH

### Gradient:

min	mL/min	%A	%B	Curve
0.00	0.5	90	10	
2.2	0.5	30	70	6
3.67	0.5	30	70	6
3.68	0.5	90	10	6

### Temperature:

40°C

### Sample:

1.Acetoaminophen 2.Guaifenesin 3.Propranolol  
 4.Diphenhydramine 5.Bromhexine 6.Ketoprofen  
 7.Flurbiprofen 8.Ibuprofen

### Injection volume:

0.2uL

### System:

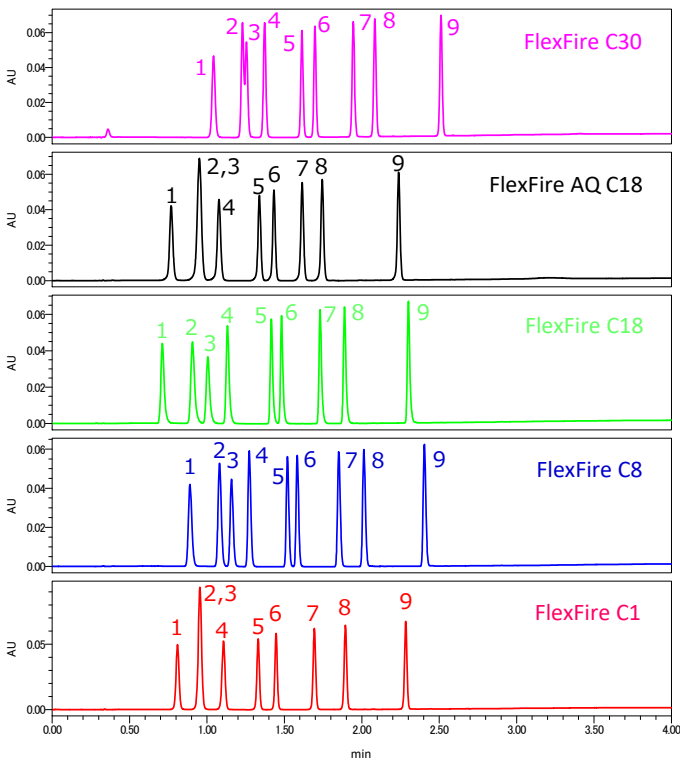
Waters ACQUITY UPLC H-Class PLUS

### Mixer:

100uL

Formic acid mobile phase can also be used for the analysis that has been performed with phosphate buffers. The combination with UHPLC accelerates the labor and analysis time further.

## Analysis of Sulfa drugs



### Conditions;

Column: FlexFire C30 1.6um (2.0x50mm)  
 FlexFire AQ C18 1.6um (2.0x50mm)  
 FlexFire C18 1.6um (2.0x50mm)  
 FlexFire C8 1.6um (2.0x50mm)  
 FlexFire C1 1.6um (2.0x50mm)  
 Mobile phase: A) Water + 0.1% HCOOH B) Acetonitrile + 0.1%HCOOH

### Gradient:

min	mL/min	%A	%B	Curve
0.00	0.5	90	10	
2.20	0.5	60	40	6
3.67	0.5	60	40	6
3.68	0.5	90	10	6

### Temperature:

40°C

### Detection:

UV275nm

### Sample:

1. Sulfadiazine (49ug/mL)  
 2. Sulfathiazole (51ug/mL)  
 3. Sulfapyridine (52ug/mL)  
 4. Sulfamerazine (52ug/mL)  
 5. Sulfamethazine (53ug/mL)  
 6. Sulfamethoxyipyridazine (50ug/mL)  
 7. Sulfachloropyridazine (52ug/mL)  
 8. Sulfamethoxazole (52ug/mL)  
 9. Sulfadimethoxine (53ug/mL)

### Injection volume:

0.2uL

### System:

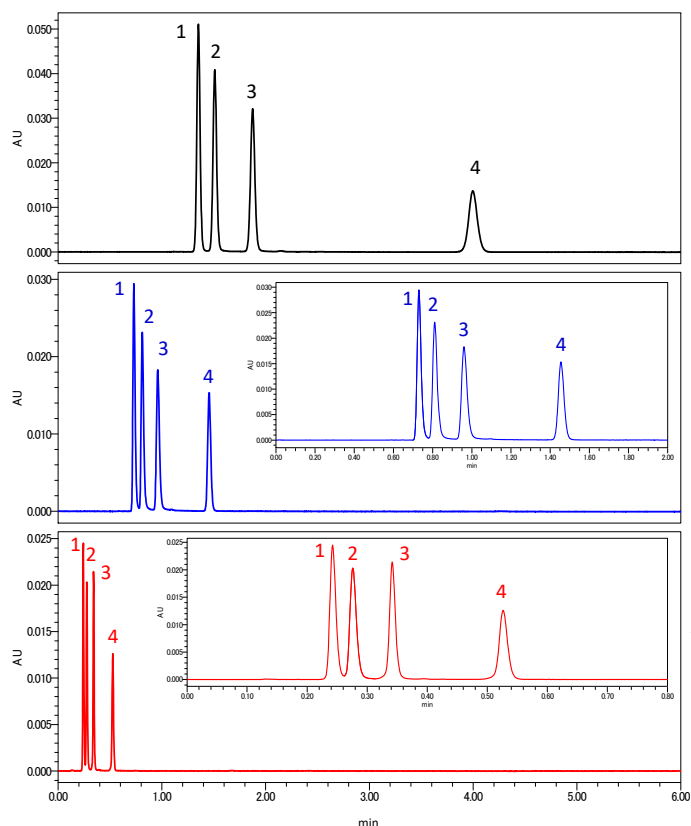
Waters ACQUITY UPLC H-Class PLUS

Analysis of sulfa drugs achieves complete separations at the high binding densities of FlexFire C18 and C8.

The method for simple and short-term analysis allows smooth column selection.



## Analysis of catecholamines



### Conditions;

**Column:** FlexFire AQ C18 5um (2.0x150mm)  
 FlexFire AQ C18 2.6um (2.0x100mm)  
 FlexFire AQ C18 1.6um (2.0x50mm)

**Mobile phase:** 0.1% HCOOH  
**Flow rate:** 5um: 0.2mL/min  
 2.6um: 0.3mL/min  
 1.6um: 0.5mL/min

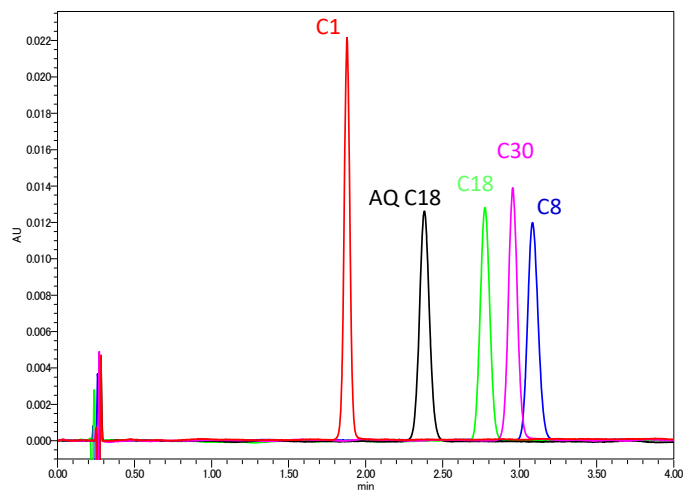
**Temperature:** 40°C  
**Detection:** UV260nm  
**Sample:** 1. Norepinephrine (98ug/mL)  
 2. Epinephrine (96ug/mL)  
 3. Dopamine (97ug/mL)  
 4. Tyrosine (98ug/mL)

**Injection volume:** 5um: 0.5uL  
 2.6um: 0.3uL  
 1.6um: 0.2uL

**System:** Waters ACQUITY UPLC H-Class PLUS

This is an example of the UHPLC method transfer of catecholamines using a 0.1% formic acid mobile phase. The HPLC method, which is completed in about 4 minutes, is completed in about 40 seconds by transferring to 1.6 µm particle UHPLC.

## Analysis of diclofenac Na



### Conditions;

**Column:** FlexFire C30 1.6um (2.0x50mm)  
 FlexFire AQ C18 1.6um (2.0x50mm)  
 FlexFire C18 1.6um (2.0x50mm)  
 FlexFire C8 1.6um (2.0x50mm)  
 FlexFire C1 1.6um (2.0x50mm)

**Mobile phase:** Acetonitrile/0.1%HCOOH=40/60  
**Flow rate:** 0.5mL/min  
**Temperature:** 40°C  
**Detection:** UV275nm  
**Sample:** 1. Diclofenac Na (99ug/mL)  
**Injection volume:** 0.2uL

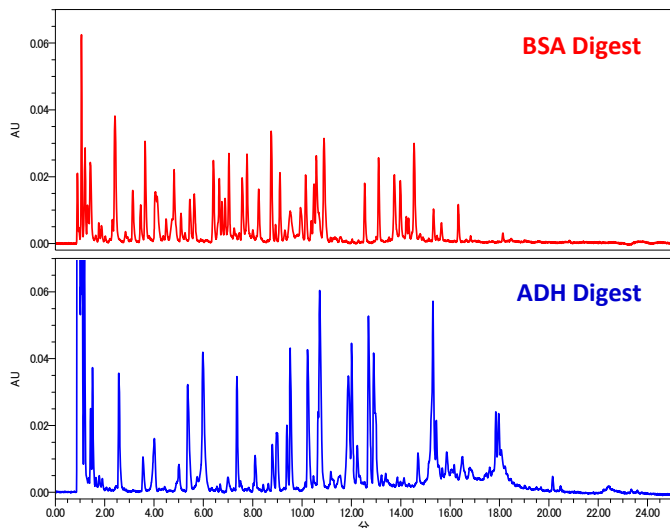
**System:** Waters ACQUITY UPLC H-Class PLUS

FlexFire C1 can be detected with high sensitivity due to its low adsorption.

We use this column for the analysis of fine components such as impurities.



## Analysis of digested proteins



### Conditions;

Column: FlexFire mAb-RP, 2.6 $\mu$ m (2.0x100mm)  
 Mobile phase: A) Water+0.1%HCOOH B) Acetonitrile+0.1%HCOOH

### Gradient:

min	mL/min	%A	%B	Curve
0.00	0.3	100	0	
30.0	0.3	50	50	6
30.1	0.3	100	0	6

Temperature: 40°C

Detection: UV210nm

Sample: BSA Digest

ADH Digest

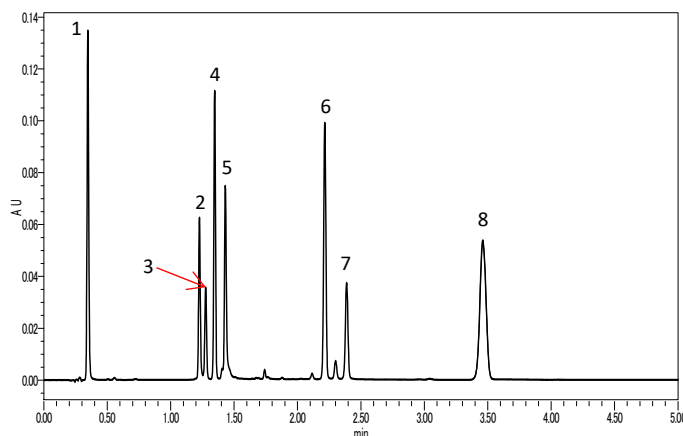
Injection volume: 10 $\mu$ L

System: Waters ACQUITY UPLC H-Class PLUS

Mixer: 100 $\mu$ L

Proteins with reduced molecular weight can achieve satisfactory results even under 0.1% formic acid conditions without using TFA.

## Analysis of polyphenols



### Conditions;

Column: Flex Fire C18, 1,6 $\mu$ m (2.0x50mm)  
 Mobile phase: A) Water + 0.1% HCOOH  
 B) Acetonitrile + 0.1% HCOOH

### Gradient:

min	mL/min	%A	%B
0.00	0.5	80	20
1.28	0.5	45	55
3.60	0.5	45	55
3.61	0.5	80	20

Temperature: 40°C

Detection: UV260nm

Sample: 1.Puerarin (10.4 $\mu$ g/mL) 2.Baicalin (10.2 $\mu$ g/mL)

3.Resveratrol (9.5 $\mu$ g/mL) 4.Daidzein (11.6 $\mu$ g/mL)

5.Quercetin (9.0 $\mu$ g/mL) 6.Biochanin A (9.7 $\mu$ g/mL)

7.Curcmin (10.0 $\mu$ g/mL) 8.Ipriflavone (11.4 $\mu$ g/mL)

Injection volume: 0.2 $\mu$ L

System: Waters ACQUITY UPLC H-Class PLUS

Mixer: 100 $\mu$ L

A simple mobile phase and UHPLC analysis conditions will give the shortest results. Above all, 0.1% formic acid is suitable for the first method because everyone knows it and it is easy to handle.

### ■ お問い合わせ/Contact us



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