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# Ammonium fluoride as suitable additive for HILIC-based LC-HRMS metabolomics

Luca NARDUZZI<sup>1</sup>, Anne-Lise ROYER<sup>1</sup>, Emmanuelle BICHON<sup>1</sup>, Yann GUITTON<sup>1</sup>, Bruno LE BIZEC<sup>1</sup>, Corinne BUISSON<sup>2</sup>, Gaud DERVILLY<sup>1</sup>



## Introduction

- Ammonium fluoride salt is becoming a popular additive in LC-MS, due to its well-known ability to increase compounds' ionization. Nevertheless, an objective evaluation of its applicability in Hydrophilic Interaction Liquid Chromatography (HILIC) is missing.
- In this study, we developed a HILIC chromatographic method using **ammonium fluoride** as additive and we compared it to a standard **ammonium acetate** method already in use in our laboratory.

## Materials and Methods

**COLUMN:** SeQuant ZIC-HILIC 200Å, 2.1\*100 mm (Merck-Millipore, Fontenay sous-Bois, France)

**Eluents A-B:** Acetonitrile-Water

**Instrument:** 1200 infinity HPLC (AgilentTechnologies, CA, USA), Exactive Orbitrap MS (ThermoFisher Scientific, Bremen, Germany)

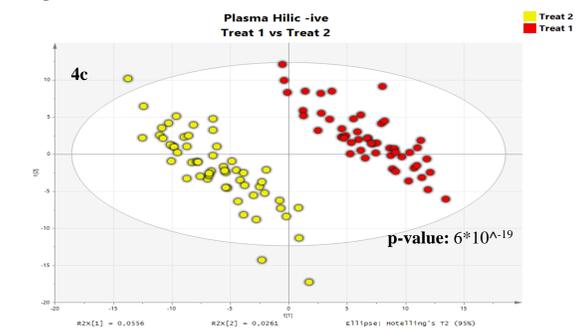
ADDITIVE SALTS:

**Ammonium Fluoride (AF) 2 mM**

**Ammonium Acetate (AA) 10 mM**

## 3. Applying AF in real metabolomics experiments

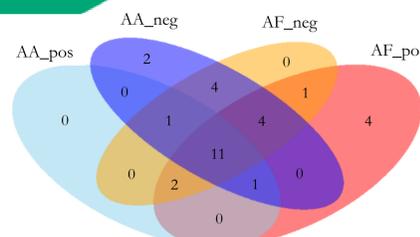
The developed AF chromatographic method has been used to perform two real metabolomics experiments with urine and plasma samples (Narduzzi et al. submitted). The method showed to be robust across 5 days of injection and the results very satisfying in terms of discriminative power (figure 4).



**Figure 4:** a PLS-DA analysis of the plasma samples injected using the AF chromatographic method.

## 2. Pooled samples injections comparison

Pooled samples of human urine have been injected using both chromatographic methods to compare the number of compounds detected, the MS signal intensity and the intra-day repeatability of the method (figure 3 and table 2)



**Figure 3:** the number of compounds detected. AF pos-neg ionize more compounds than AA in real urine samples.

|                  | Ammonium Acetate     |                      | Ammonium Fluoride     |                      |
|------------------|----------------------|----------------------|-----------------------|----------------------|
|                  | ESI+                 | ESI-                 | ESI+                  | ESI-                 |
| additive         |                      |                      |                       |                      |
| ND** peaks       | 14                   | 11                   | 7                     | 11                   |
| Median peak area | 6,9*10 <sup>-5</sup> | 6,6*10 <sup>-5</sup> | 1,05*10 <sup>-6</sup> | 2,9*10 <sup>-6</sup> |
| Median area      | 29                   | 17                   | 10                    | 8.7                  |
| RSD*             | max-min 49-8         | max-min 90-6         | max-min 53-2.8        | max-min 45-5         |
| Mean RT shift    | 11 s                 | 16 s                 | 10 s                  | 9 s                  |

**Table 2:** Comparison of the method performances in terms of number of non-detected peaks (ND), median peak area, median relative standard deviation (RSD), and mean retention time shift (RT shift).

Find out the advantages of AMMONIUM FLUORIDE in HILIC-MS!

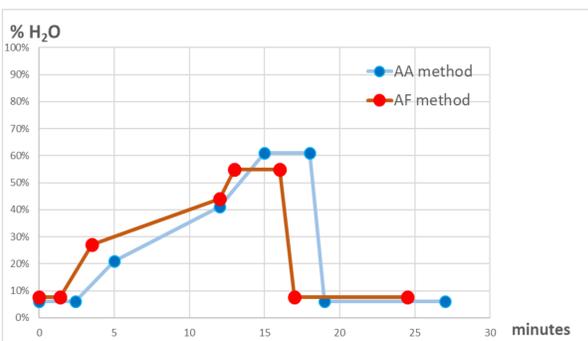


SCAN ME

Narduzzi, L. et al. Ammonium Fluoride as Suitable Additive for HILIC-Based LC-HRMS Metabolomics. *Metabolites* 9(12), 292 (2019), .

## 0. Chromatographic method development

Due to the different ionic strengths, specific chromatographic methods have been developed for each additive as reported in figure 1. The AF and AA methods are long 24,5 and 27,5 minutes long respectively.



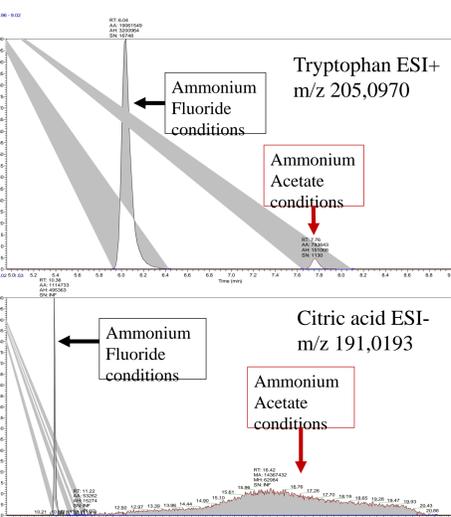
**Figure 1:** Schematic representation of the two chromatographic methods.

## 1. Standards ionization comparison

34 analytical standards representing various chemical classes have been injected in both methods to compare the ionization performances. The AF method resulted most performing in both positive and negative ionization modes (figure 2 and table 1).

|             | Ammonium Acetate          |                       | Ammonium Fluoride     |                       |
|-------------|---------------------------|-----------------------|-----------------------|-----------------------|
|             | ESI+                      | ESI-                  | ESI+                  | ESI-                  |
| median area | 5,2*10 <sup>-6</sup>      | 10,4*10 <sup>-6</sup> | 42,1*10 <sup>-6</sup> | 35,5*10 <sup>-6</sup> |
| ESI+ AF/AA  | <b>8.1 folds increase</b> |                       |                       |                       |
| ESI- AF/AA  | <b>3.5 folds increase</b> |                       |                       |                       |

**Table 1:** a comparison of the median peak area of the 34 standards used in this experiment in both ESI positive and negative ionizations.



**Figure 2:** Comparison of the peak size of Tryptophan and Citric acid with AF vs AA. Both compounds have been injected using the methods depicted in figure 1.

## Conclusion and perspectives

Ammonium fluoride showed higher ionization efficiency, stability and repeatability than ammonium acetate in HILIC-MS experiments. These characteristics make ammonium fluoride as a candidate additive in metabolomics experiments. Performing two real metabolomics experiments, we achieved good stability and a good discriminant power, without having troubles during the sequence injections. Future work should focus on the determination of the best ammonium fluoride concentration specific for each kind of chromatographic column and instrument.

