

Development and optimization of a derivatization protocol for phenethylamine compounds using FITC-CE-LIF method

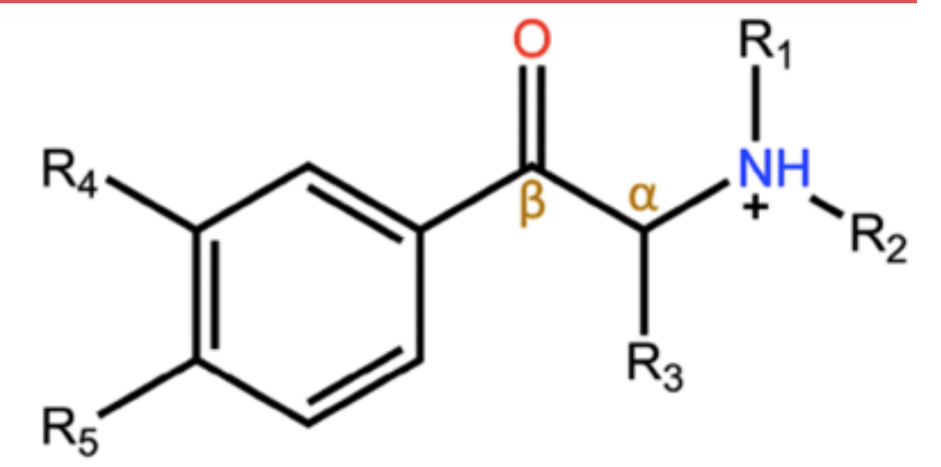
Paul Emonts, Caroline Ninane, Philippe Hubert, Anne-Catherine Servais, Eric Ziémons, Marianne Fillet, Amandine Dispas
Laboratory for the Analysis of Medicines
Laboratory of Pharmaceutical Analytical Chemistry
CIRM, University of Liège, Avenue Hippocrate 15, 4000 Liège

INTRODUCTION

Synthetic cathinones (SCs) are compounds from the phenethylamine family. These compounds are mainly drugs of abuse because of their close structure with amphetamine. SCs were the second most frequently seized group of new psychoactive substances in EU in 2016. Moreover some of them are currently sold on internet as bath salts without any legislation!

In this context, we are developing a microfluidic system based on capillary electrophoresis separation with a Laser Induced Fluorescence (LIF) detection in order to track and quantify these compounds. Indeed this innovative system will provide some advantages in terms of sensitivity, speed, easiness and compact size compared to existing systems.

This study presents first optimization of a derivatization protocol of phenethylamine compounds (and derivatives) as model compounds, using Fluorescein IsoThioCyanate (FITC). Moreover, a CE-LIF method was developed in parallel in order to follow up this first optimization, and in the future, to compare analytical performances of traditional CE-LIF and microfluidic CE-LIF system.



MATERIAL AND METHOD

Model Compounds

Adrenaline (A) – Phenylephrine (Ph) – Pseudoephedrine (Ps) – Dexamphetamine (D) – Metoprolol (M)

Derivatization Protocole

Reaction and dilution medium : Borate buffer 20 mM pH10
FITC dissolved in DMSO/pyridine 99.5/0.5
Ratio 20 : 1 (FITC : analyte)
Reaction during 4h à 5°C

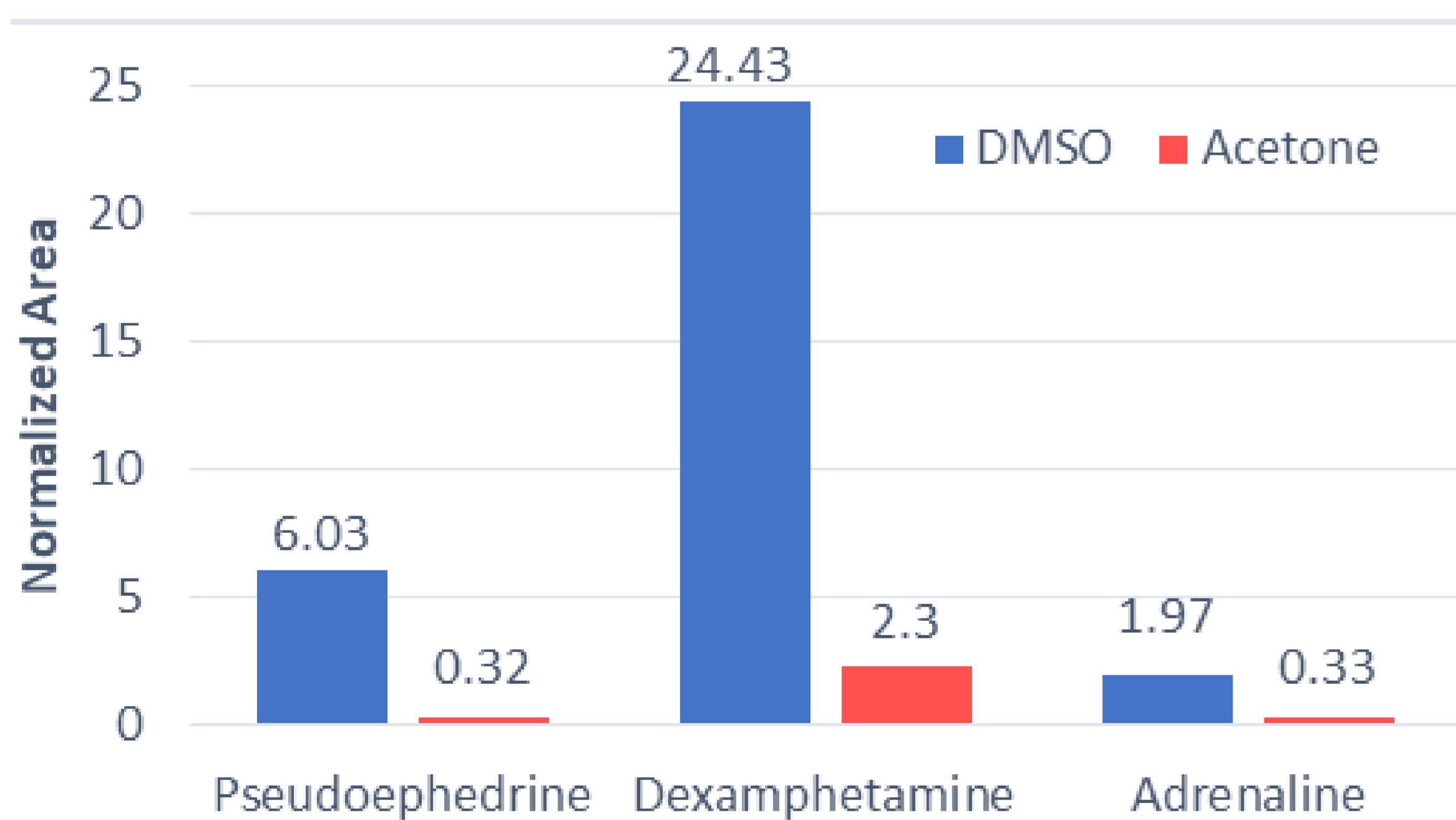
CE-LIF

Agilent 7100 Capillary Electrophoresis
Picometrics ZETA LIF detector using a 488 nm laser (20 mW)
Silica capillary 50 µm id, 38,5 cm effective length (59 cm total length)
Injection : 50 mbar during 20s ; Voltage : 25 kV
BGE : 100 mM acetate ammonium buffer at pH 9 + 10% MeOH
Target concentration 10⁻⁸ M

DERIVATIZATION OPTIMIZATION

FITC Dissolution Medium

- Acetonitrile - Acetone – DMSO – Ethanol
- With pyridine at 0 - 0.2 - 20%



Nature of Reaction Buffer

- Ammonium acetate
- Borate

Temperature

- 5°C – 25°C – 40°C
- Degradation of FITC

pH of Reaction Buffer

- 8 – 9 – 10
- Deprotonated amine (pKa!)
- Stability of labeled compounds

Reaction Time

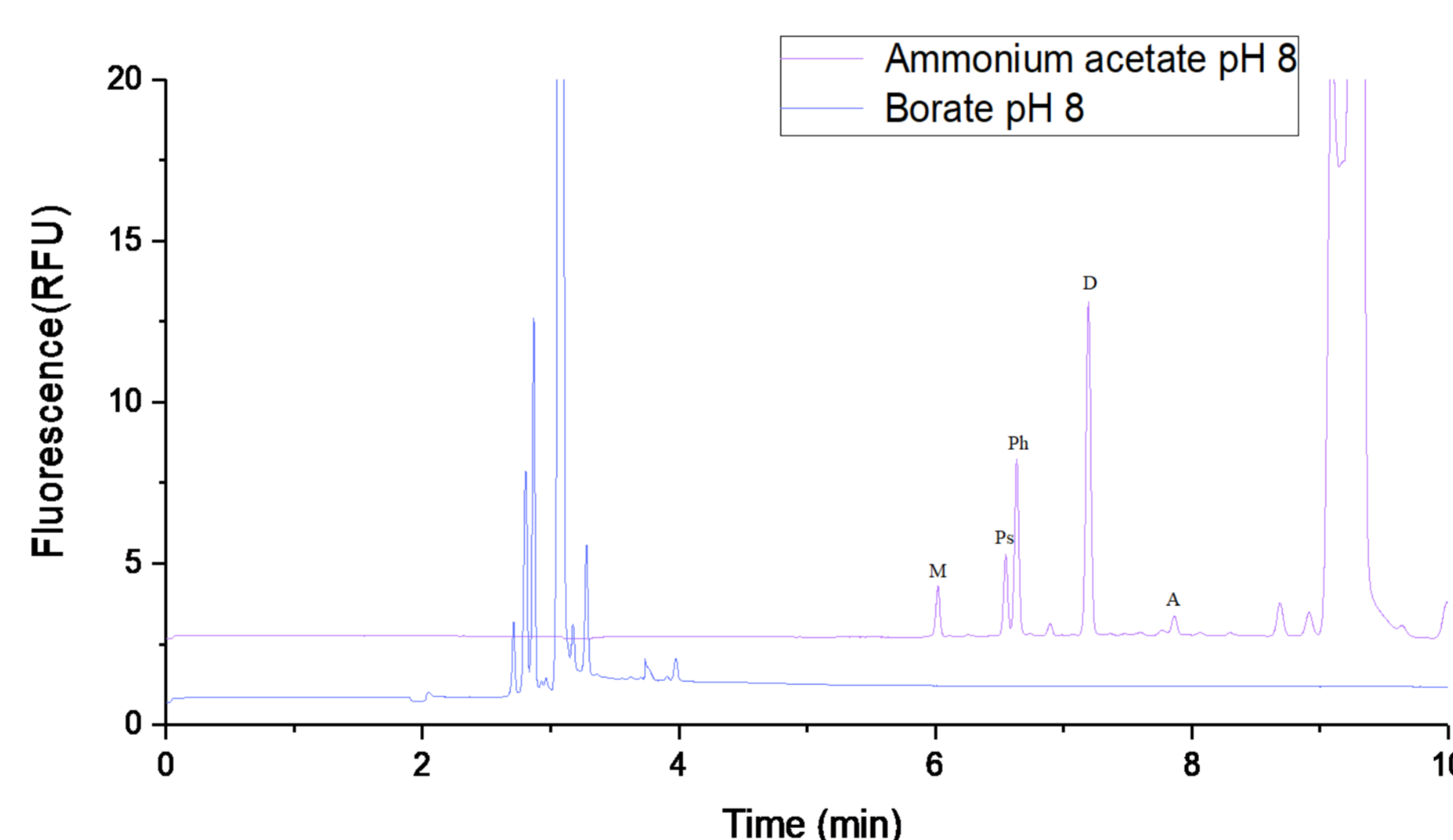
- 4h – 8h – 24h
- Signal intensity comparable
- Technical constraints

SEPARATION OPTIMIZATION

BGE Ionic Strength 25 – 50 – 100 mM

BGE buffer nature

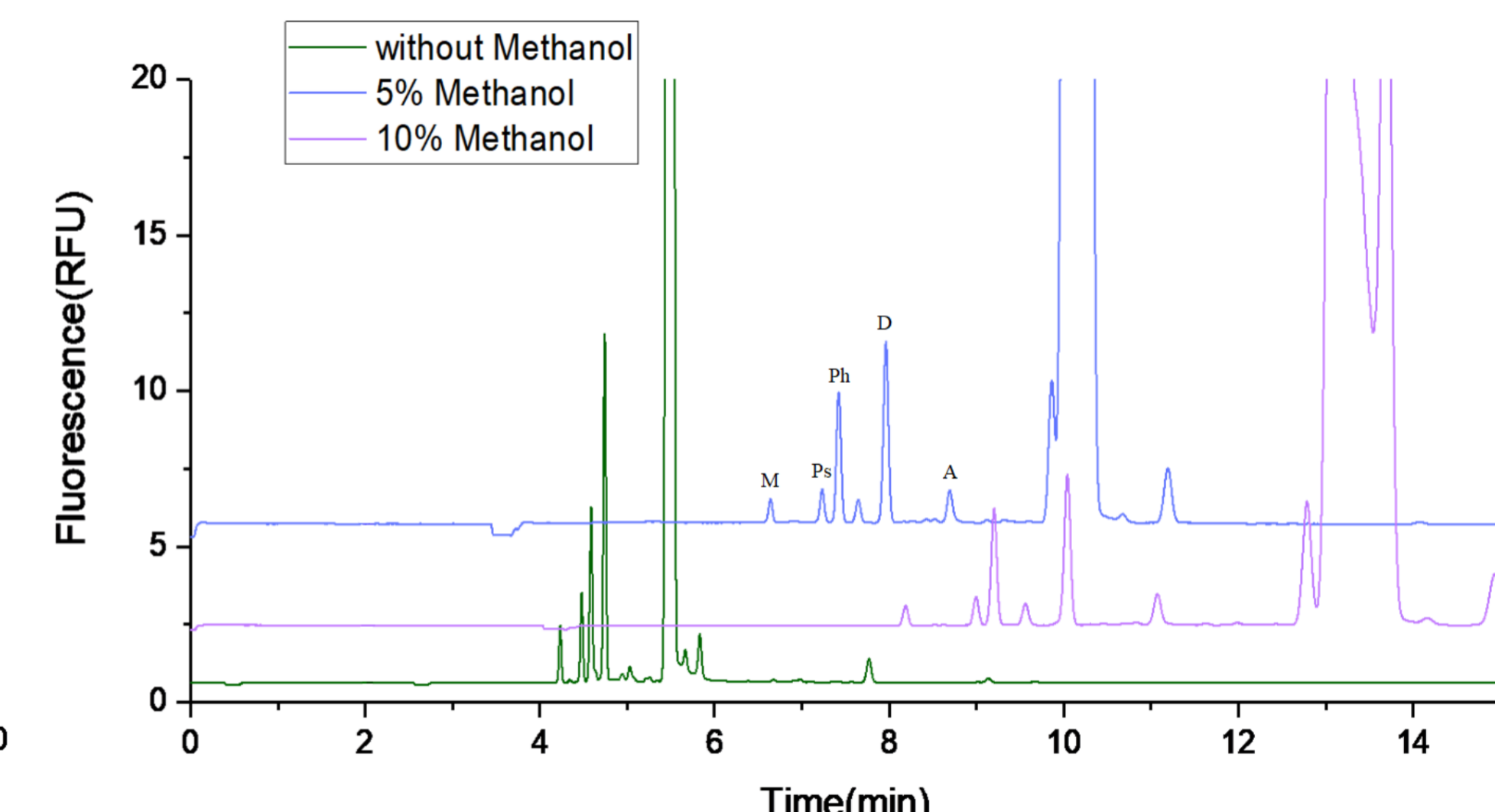
Ammonium acetate - Borate



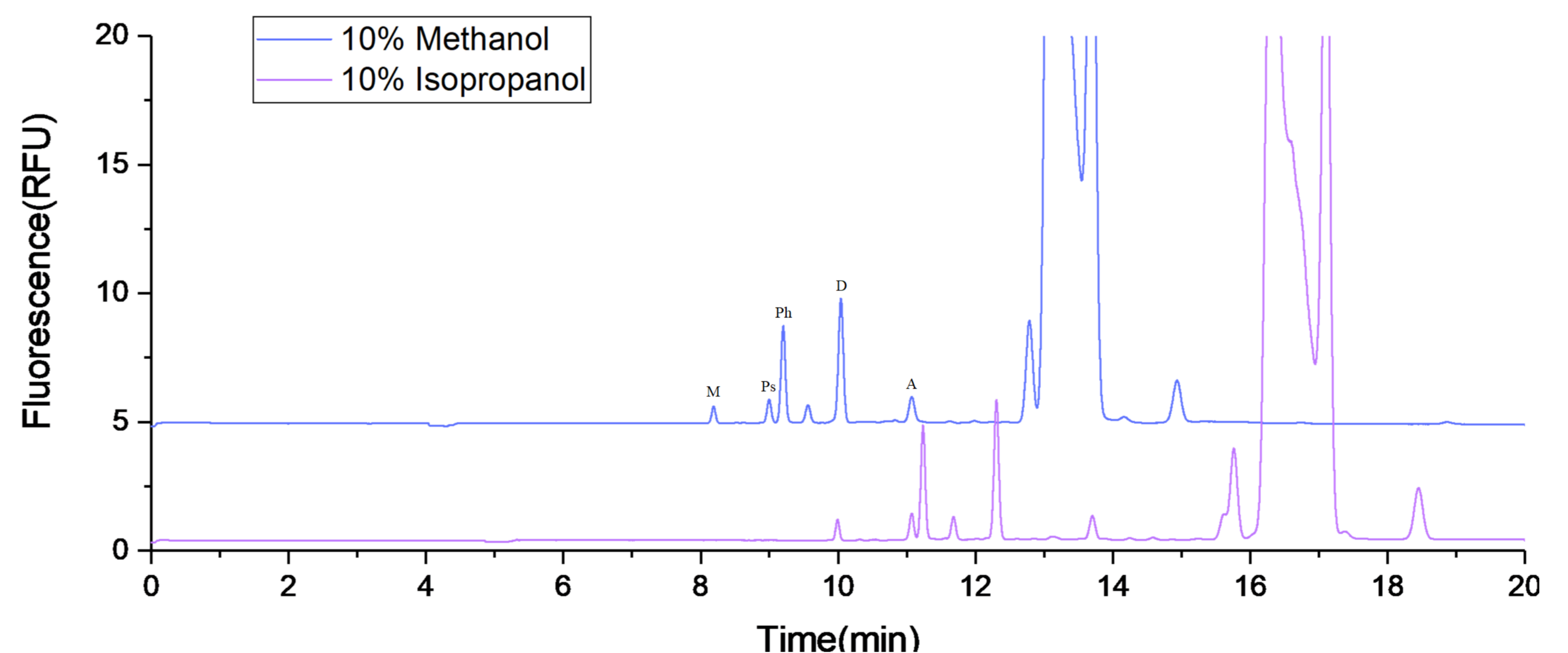
pH 8 – 9 (one co-migration at pH 8)

BGE additive %

0 – 5 – 10 % Methanol



BGE Additive nature Methanol - Isopropanol



CONCLUSIONS AND PERSPECTIVES

- Selection of critical method parameters to maximize **derivatization yield** on phenethylamine compounds
 - **Design of experiment** strategy will be used to optimize the protocol for future SCs derivatization
- Development of a CE separation method for phenethylamine analysis using a **sensitive** LIF detection
 - Separation of **closely related structure** compounds
 - Relatively large migration window for future applications
 - Both isopropanol and methanol can be used
 - Utilization of a **MS compatible BGE** for future application as new SC derivatives HR-MS identification
 - CE-LIF developed method will be transferred and adapted to the microfluidic system
 - **Comparison of analytical performances** of traditional CE-LIF vs µCE-LIF systems