Analysis of metabolites in ionic exchange chromatography coupled to mass spectrometry
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To cite this version:
Adeline Bigard, Adeline Clergé, Nathalie Hebert, Pascal Cardinaël, Valérie Peulon-Agasse. Analysis of metabolites in ionic exchange chromatography coupled to mass spectrometry. 36th International Symposium on Microscale Separations and Bioanalysis (MSB 2020), Apr 2020, Saint Malo, France. hal-03560102

HAL Id: hal-03560102
https://normandie-univ.hal.science/hal-03560102
Submitted on 21 Feb 2022

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Introduction

- Cancers: 382,000 new cases in France in 2018.1
- Neurodegenerative diseases: continually increase due to population ageing (220,000 new cases of Alzheimer’s disease / year)2.
- Dysfunction in energy cycles such as Krebs cycle or nucleoside catabolism: sources of different diseases.

Anomalies → disrupt metabolic balances → modulation of cellular epigenetics → tumor process engagement
- To facilitate an early diagnosis and propose an appropriate treatment: detection and quantification of biomarkers.
- In this work, an IC-MS/MS method was developed for future metabolomics analysis.

Device and method

**Ion chromatography coupled with triple quadrupole mass spectrometer**

| Ion chromatography conditions: | Column: AS11-HC4-mu bonded C4imm
| | Mobile phase: NaOH 0.1M
| | Injected volume: 10 µL
| | Flow: 0.3 mL/min
| | Column T^*: 21°C
| | Suppressor: 75 mA
| | Gradient: 0 min 5 mM
| | 30 min 100 mM
| | 50 min 500 mM
| | Make-up flow (MeCN): 0.1 mL/min

**Mass spectrometry conditions:**

| Source | ESI
| | Neg. Ion Spray | 2000 V
| | Steam Gas | 23 Arb
| | Aux. Gas | 10 Arb
| | Sweep Gas | 1.5 Arb
| | Suppressor T^* | 150°C
| | Ion Transfer T^* | 300°C

Optimisation

With IC-MS/MS, some parameters should be optimized to detect charged compounds in conductivity cell and in MS. In this purpose, the suppression current and the make-up solvent were optimized. The make-up solvent could be modified but also its composition and its rate. Example of optimization realized on phosphate compounds.

**Choice of make-up solvent**

- Two different make-up solvents were tested: methanol and acetonitrile. A good make-up solvent should improve sensitivity and make the spray as stable as possible.
- MeCN: best solvent allowing an increase of the sensitivity.
- Stability of the ion spray: determination of the coefficient of variation (CV) based on four replicates.
- McAcN: gave the most stable ion spray.
- The choice was also made according to the background noise which must remain low.
- Analysis were realized with MeCN as make-up solvent.

**Choice of make-up percentage**

- Four rates of McAcN were tested: 0 %, 30 %, 50 % and 75 % to optimize the ionization in mass spectrometry.
- A compromise between sensitivity and background noise has to be done.
- The best compromise correspond to 30 % of McAcN.

Assay in real matrix

To study applicability of the optimized method (with 30% of McAcN, a suppression current of 75 mA and an analysis at pH 6), first assays were carried out in saliva and urine on the 52 selected compounds. The main interest of this method was to overcome sample preparation step.

**Example of the urine in real matrix**

- 28 compounds of our mixture were previously described in urine.
- Urine was diluted by 2 before injection.

**Urine**

23/28 compounds were observed in urine.

**Saliva**

27 compounds of our mixture were previously described in saliva.
- Saliva was diluted by 2 before injection.

16/27 compounds were observed in saliva.

**Water**

52 compounds in our mixture.
- Compounds around 0.4 mM.

41/52 standards were observed in water.

Conclusion

- Ion chromatography method coupled with mass spectrometry has been optimized.
- This technique allowed to overcome sample preparation step.
- Most compounds were detected in saliva and urine.
- This IC-MS/MS method was developed for future metabolomics analysis.

Perspectives

- Optimisation of the non-detected compounds.
- Validation of the optimized IC-MS/MS method.
- Study of the matrix effect.
- Quantification of the 52 compounds in biological matrices.