Analysis of metabolites in ionic exchange chromatography coupled to mass spectrometry

Adeline Bigard, Adeline Clergé, Nathalie Hebert, Pascal Cardinaël, Valérie Peulon-Agasse

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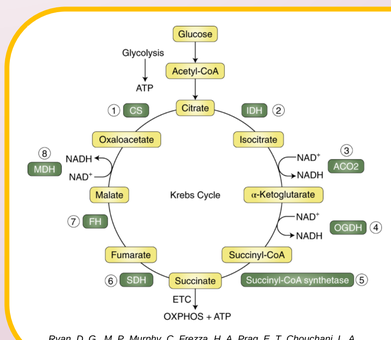
Introduction



Ryan, D. G., M. P. Murphy, C. Frezza, H. A. Prag, E. T. Chouchani, L. A. O'Neill, and E. L. Mills. Nature Metabolism 1, no. 1 (January 2019): 16–33.

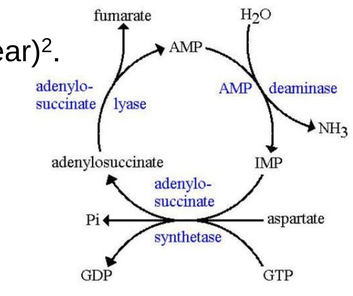
- Cancers: 382 000 new cases in France in 2018[1].
- Neurodegenerative diseases : continually increase due to population ageing (220 000 new cases of Alzheimer's disease / year)[2].
- Dysfunction in **energy cycles** such as Krebs cycle or nucleoside catabolism : sources of different diseases[3].

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**.

→ 52 **small acids** and **phosphate compounds** were selected.

- In this work, an **IC-MS/MS method** was developed for future metabolomics analysis.



Bhagavan, N. V. "CHAPTER 27 - Nucleotide Metabolism." Medical Biochemistry (Fourth Edition), 615–44. 2002.

Device and method

Ion chromatography coupled with triple quadrupole mass spectrometer

Ion chromatography conditions :

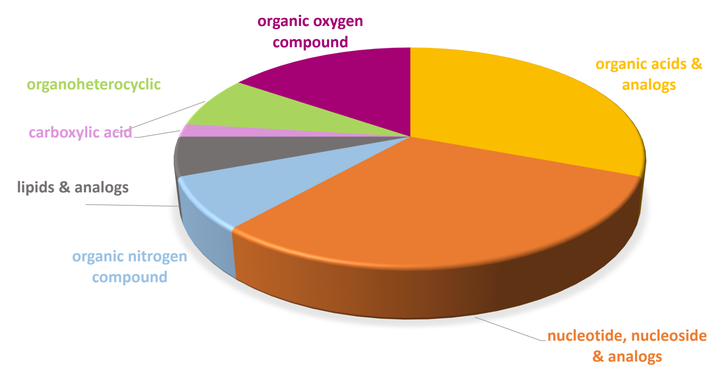
Column	AS11-HC-4µm 2x250mm
Mobile phase	NaOH 0.1M
Injected volume	10 µL
Flow	0.3 mL/min
Column T°	21°C
Suppressor	75 mA
Gradient	0 min 5mM 30 min 100 mM 40 min 100 mM 40.1 min 5 mM 50 min 5mM
Make-up flow (MeCN)	0.1 mL/min

Mass spectrometry conditions :

Source	ESI
Neg. Ion Spray	2800 V
Sheath Gas	29 Arb
Aux. Gas	10 Arb
Sweep Gas	1,5 Arb
Vaporizer T°	150°C
Ion Transfer T°	300°C

Analytes of interest

Classes of analyzed compounds

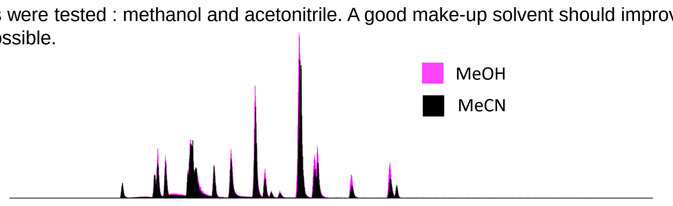


Optimisation

With IC-MS/MS, some parameters should be optimized to detect charged compounds in conductimetry 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.



Comparison of intensity of signal with TIC of MeOH and MeCN

- MeOH : best solvent allowing an increase of the sensitivity.
- Stability of the ion spray : determination of the coefficient of variation (CV) based on four replicates.

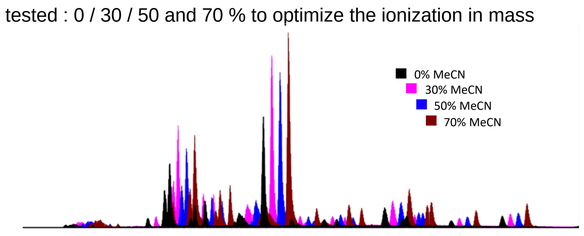
	MeOH	MeCN
CV min (%)	5.5	0.7
CV max (%)	196.8	108.2
CV average (%)	30.7	16.7

- MeCN : gave the most stable ion spray.
- The choice was also made according to the backpressure which must remain low.

→ Analysis were realized with MeCN as make-up solvent.

Choice of make-up percentage

- Four rates of MeCN were tested : 0 / 30 / 50 and 70 % to optimize the ionization in mass spectrometry.



TIC of the mixture with different percentages of acetonitrile (time offset)

- A compromise between sensitivity and backpressure has to be done.

→ The best compromise correspond to 30 % of MeCN.

Analytes

- **26 phosphated compounds** (nucleotides and organic oxygen compounds essentially).
- **Concentration about 20 mg/L** (enough concentrate to detected all components including those with weak signals).

Conditions

- Conditions described above.
- **SRM Mode** with optimized transitions for each compound.

Influence of suppression current and pH before ionization

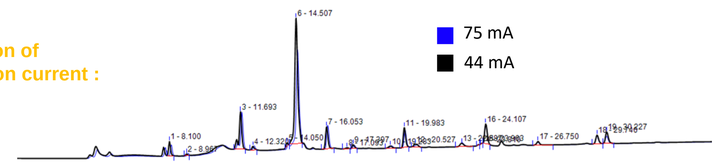
- Some compounds are difficult to detected in conductimetry and/or in MS.
- Example of Phosphoethanolamine (PE) :

pK_a and state of charge of phosphoethanolamine

→ pH < 6.51, PE is **neutral or positive** and can't be analyzed in this conditions.

- Technically : the role of the suppressor was to neutralize the mobile phase before conductimetry analysis (pH must be around 7 and compounds must be charged). At this pH, PE should be detected.
- Experimentally : the suppressor is too efficient. pH is approximately at 5.5 before addition of the make-up solvent. That could explain the fact that PE is not observed in conductimetry. pH is near to 6 after adding the MeCN.

Modification of suppression current :



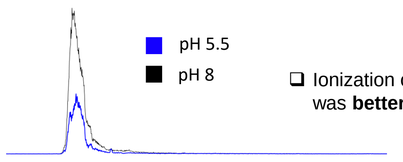
Comparison of analysis with a suppressor current of 75 mA and 44 mA.

- A decrease of the suppression current leads to similar sensitivity (with the exception of an increase for the peak at 14.507 min which correspond to cAMP, dAMP and NADH).
- The recommended current for the chosen gradient was 75 mA.

→ Retain a suppression current of 75 mA.

Modification of pH :

- pH is modified by the addition of ammonium hydroxide into the make-up solvent.



Example of PE signal at pH 5.5 and at pH 8

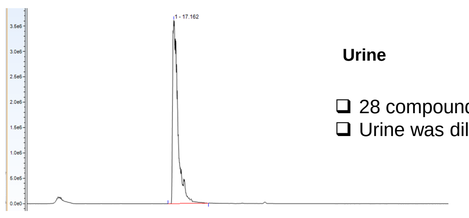
- Ionization of the different compounds and intensity of signal was **better at pH 8.**

→ Selection of pH 8 in make-up solvent.

Assay in real matrix

To study applicability of the optimized method (with 30% of MeCN, a suppression current of 75 mA and an analysis at pH 8), 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**.

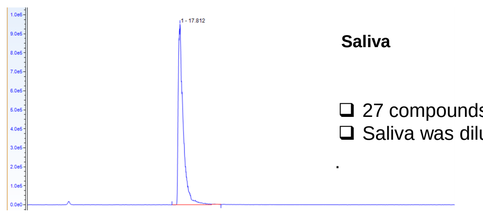
Example of the urate in real matrix



Urine

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

→ 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.

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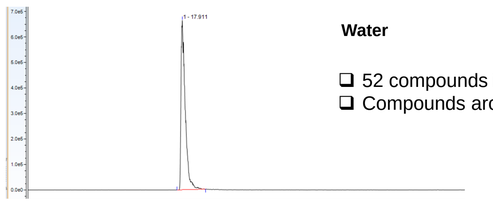
→ 16/27 compounds were observed in **saliva.**



Water

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

→ 41/52 standards were observed in **water.**

Ion chromatogram of urate in urine, saliva and 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/SM 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**.

1. https://www.santepubliquefrance.fr
2. https://institutducerveau-icm.org/fr .
3. P. Bénit, S. Bortoli, D. Chrétien, M. Rak and P. Rustin, "Pathologies liées aux déficits du cycle de Krebs", *Revue Francophone des laboratoires*, n°501, April 2018.