# The potential of LC-MS in metabolomics of serum and plasma samples

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## Introduction

**Metabolomics**= comprehensive analysis of all low molecular weight compounds (< 1,5 kDa), metabolites, in a sample such as cells, biofluids and organisms (1).

There are **two main approaches** in metabolomics, targeted and untargeted metabolomics. **Targeted** metabolomics focuses on quantification of a pre-determined set of known metabolites while **untargeted** metabolomics aims to provide the widest possible metabolome coverage.

**Sample preparation** should enable extraction of the largest number of metabolites without introducing any kind of bias towards chemical families.

The most popular platform for sample analysis in metabolomics is **LC-MS** because it provides the highest metabolite coverage and has the capacity to separate a wide range of metabolites before detection (1,2).

The **aim** is to determine if and what differences are present between serum and plasma, how different sample preparation methods might influence the amount and chemical properties of metabolites recovered during untargeted metabolomics and the potential of LC-MS analysis to detect different chemical groups at different concentrations ranging from mM to µM.

## Materials en methods

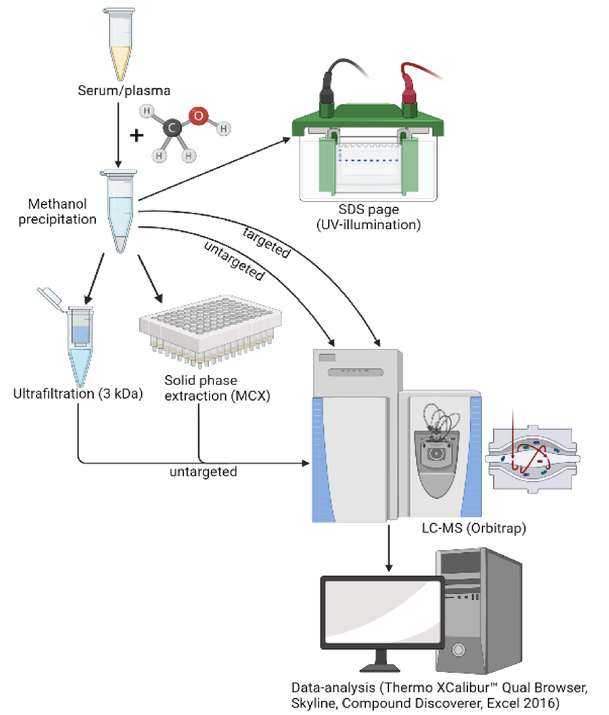


Figure 1: Overview of materials and methods (Created with BioRender.com)

## Results

### Sample preparation: protein removal and testing LC-MS effectiveness

Comparing the sample to the standard curve (Figure 2) shows that the sample after methanol precipitation has approximately the same intensity as the original sample 200X diluted. However, there are still more small proteins left in the sample than in the 200X dilution, approximately the same intensity as the 100X dilution.

To determine the effectiveness of LC-MS, both matrices were evaluated after different purification methods (Figure 3). Kynurenine was more present in serum than plasma and > 70% was lost during ultrafiltration. Glutamate on the other hand, is more present in both matrices after ultrafiltration.

SPE, even though a very selective purification method, proved most useful for kynurenine. Glutamate almost fully lost during the washing steps and would not benefit from SPE. (Table 2)

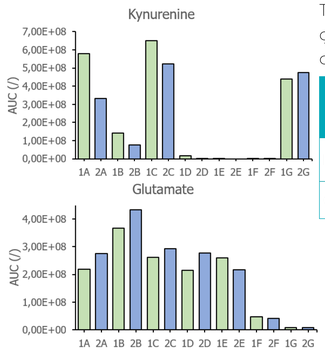


Figure 3: Comparison of different compounds from the internal control in serum (1) and plasma (2) samples after different purification methods

Table 2: Loss of kynurenine and glutamate in serum and plasma after solid phase extraction (SPE)

| Molecule | Loss in serum (%) | Loss in plasma (%) |
|---|---|---|
| Kynurenine | 32,3 | 8,7 |
| Glutamate | > 95% | |

A + C – After methanol precipitation
B – After ultrafiltration
D – Sample flow through SPE
E – Wash step 1 SPE
F – Wash step 2 SPE
G – Elution step SPE

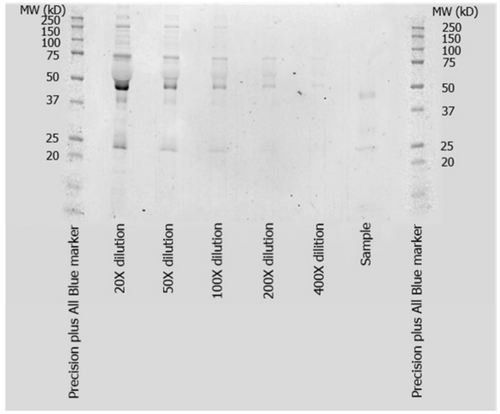


Figure 2: SDS page for quantification of proteins present in samples after methanol precipitation

Table 1: Different chemicals from standard curves with the LOQ and the area at the LOQ

| Chemical | Chemical group | Limit of quantification (LOQ) | Area at LOQ (/) |
|---|---|---|---|
| Glucose | Monosaccharide | 100 mM | $1{,}33 \cdot 10^{5}$ |
| L- glutamic acid | Amino acid | < 1,69 µM | $1{,}18 \cdot 10^{8}$ |
| L-methionine | | < 1,69 µM | $1{,}27 \cdot 10^{8}$ |
| $NAD^+$ | Dinucleotide | < 1,69 µM | $8{,}55 \cdot 10^{6}$ |
| NADH | | 1,69 µM | $1{,}20 \cdot 10^{6}$ |
| Sodium butyrate | Short-chain fatty acid | 100 mM | $6{,}42 \cdot 10^{6}$ |

### Targeted: ability of LC-MS to detect different molecules in a concentration range

After LC-MS analysis, the limit of quantification (LOQ) for the six standard curve chemicals was determined (Table 1).

For the standard curve of NADH, the raw data is visualised in Figure 4. The peaks from the four highest concentrations got broader and the peak from the highest concentration is not a nice peak. This is also visible in the linearity ($R^2$ = 0,9125) of the standard curve (Figure 5). This poor linearity can be explained by the fact that the higher detection limit is reached between 33,3 mM and 100 mM. The linearity is the best when the starting concentration was 33,3 mM ($R^2$= 0,9837).

The lowest concentration, 1,69 µM, represents the LOQ. There is noise visible in the chromatogram (Figure 4) but there is still a nice peak detected at this concentration. At a lower concentration, the peak would not be distinguishable from the noise.

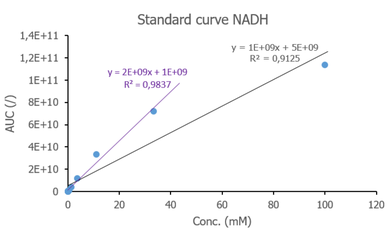


Figure 5: Standard curve NADH (100 mM - 1,69 µM)

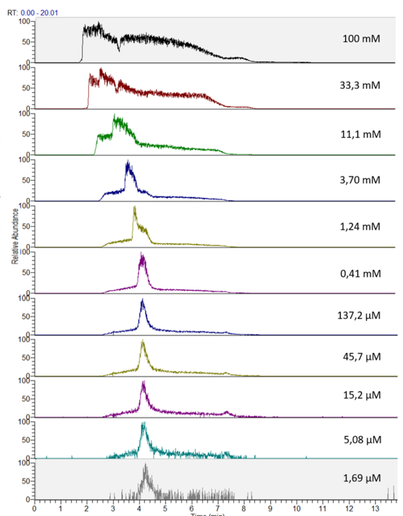


Figure 4: Raw data (chromatogram) from NADH standard curve

### Untargeted: how many metabolites can be detected?

Using Compound Discoverer, different metabolites were identified in the samples after methanol precipitation and ultrafiltration and SPE (Table 2).

Additional pruficiation methods generally decrease the number of molecules recovered in the samples but 21,9% and 17,5% of molecules in serum and plasma respectively showed an increased abundance after these additional steps. For example, L-valine is still present after shows an increased abundance after additional purification steps. This molecules would benefit from these purification steps because it experiences less disturbance by the matrix effect after these additional steps.

Table 2: Comparison of the influence of different purification methods on the metabolome size and chemical groups detected in an untargeted analysis

| | After methanol precipitation | After ultrafiltration and SPE | Difference (%) |
|---|---|---|---|
| Metabolites | 1106 | 137 | 87,6 |
| Amino acids | 18 | 9 | — |

## Conclusion

- Slight differences between serum and plasma in terms of abundance of some molecules but both useful in targtered and untargeted metabolomics
- Methanol precipitation is a good sample preparation method because it is able to remove >99% of the proteins present in serum and plasma samples and allows detection of a lot of metabolites (1 106).
- Additional purification steps, ultrafiltration and SPE, could be useful because they decrease the matrix effect for some molecules but testing should be performed to determine their worth for the molecules of interest
- LC-MS and its used settings:
  - Able to detect different chemical groups in a concentration range with a factor 1 000 (targeted)
  - Able to detect lot of metabolites (untargeted)

## References

1. Kuehnbaum NL, Britz-McKibbin P. New advances in separation science for metabolomics: resolving chemical diversity in a posgenomic era. Chem Rev. 2013 Apr 10; 113(4):2437-68.
2. Vuckovic D. Current trends and challenges in sample preparation for global metabolomics using liquid chromatography-mass spectrometry. Anal Bioanal Chem. 2012 Jun; 403(6):1523-48